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Density Levels of Pathogenic Organisms in Municipal Wastewater Sludge
A Literature Review

Camp, Dresser and McKee, Inc.
Boston, MA

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DENSITY LEVELS OF PATHOGENIC ORGANISMS IN MUNICIPAL WASTEWATER SLUDGE

A LITERATURE REVIEW

by

Dana C. Pedersen
Camp Dresser & McKee Inc.
Boston, Massachusetts 02108

Contract No. 68-03-2803

Project Officer

Gerald Stern
Wastewater Research Division
Municipal Environmental Research Laboratory
Cincinnati, Ohio 45268

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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16. ABSTRACT

This report presents a critical review of the literature from laboratory and full scale studies regarding density levels of indicator and pathogenic organisms in municipal wastewater sludges and septage. The effectiveness of conventional municipal sludge stabilization processes (mesophilic anaerobic and aerobic digestion, composting and lime stabilization) and dewatering processes (drying beds, lagooning/storage, and sludge conditioning/mechanical dewatering) was evaluated for reducing density levels of indicator and pathogenic organisms. To accomplish this objective, a comprehensive review was performed of all literature published on this topic between 1940 and 1980. An annotated bibliography presents all citations reviewed, with pertinent abstracts and methods used by researchers.

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution; it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and provides a most vital communications link between the researcher and the user community.

This report presents the state of knowledge regarding density levels of indicator and pathogenic organisms, enteroviruses, and parasites in raw, stabilized, and dewatered municipal wastewater sludges. With a better understanding of the magnitude of these agents, regulations can be developed to control these potentially hazardous materials.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

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ABSTRACT

This report presents a critical review of the literature published between 1940 and 1980 from laboratory and full scale studies regarding density levels of indicator and pathogenic organisms in municipal wastewater sludges and septage. The effectiveness of conventional municipal sludge stabilization processes (mesophilic anaerobic and aerobic digestion, composting and lime stabilization) and dewatering processes (drying beds, lagooning/storage, and sludge conditioning/mechanical dewatering) was evaluated for reducing density levels of indicator and pathogenic organisms. An annotated bibliography presents all citations reviewed, with pertinent abstracts and methods used by researchers.

This report was submitted in fulfillment of Work Task 08 for Contract No. 68-03-2803 by Camp Dresser & McKee Inc., under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period January 1980 to May 1980, and work was completed as of December 1980.

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SECTION 1

INTRODUCTION

BACKGROUND -- CRITERIA FOR SLUDGE DISPOSAL

In September 1979, the U.S. Environmental Protection Agency (EPA) promulgated 40 CFR Part 257, an interim final regulation* setting forth criteria for classifying solid waste disposal facilities and practices. The criteria are intended to distinguish clearly between the facilities and practices that conform -- and those that fail to conform -- to requirements of the Solid Waste Disposal Act, as amended by the Resource Conservation and Recovery Act (RCRA) of 1976, and of the Clean Water Act of 1977, PL 95-217.

Section 405 of the Clean Water Act requires EPA to provide guidelines for the disposal and utilization of wastewater treatment plant sludge. Therefore, in partial fulfillment of Section 405, the criteria contained in 40 CFR Part 257 provide minimum requirements to be met by any owner or operators of a publicly owned treatment works (POTW) when disposing of sludge on the land.

In promulgating 40 CFR Part 257 Criteria, EPA recognized that some portions would benefit from additional public scrutiny and comment. EPA chose to promulgate these portions of the regulations in "interim final" form --fully enforceable as published, but open to modification if warranted. Criteria relating to control of infectious disease in the land application of sewage sludge and septic-tank pumpings, a portion of Section 257.3-6, were published in this interim final form.

The interim final criteria contained in Section 257.3-6 established the minimum processing of sewage sludge and septic tank pumpings required before land application of the materials. At a minimum, one of the following "Processes to Significantly Reduce Pathogens" is required for sewage sludge applied to the land:

- o Aerobic digestion: The process is conducted by agitating sludge with air or oxygen to maintain aerobic conditions at residence times ranging from 60 days at 15°C to 40 days at 20°C, with a volatile solids reduction of at least 38%.

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*U.S Environmental Protection Agency, 40 CFR Part 257. Criteria for Classification of Solid Waste Disposal Facilities and Practices. Federal Register Vol. 44, No. 179. Thursday, September 13, 1979.

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- Air drying: Liquid sludge is allowed to drain and/or dry on underdrained sand beds, or paved or unpaved basins in which the sludge is at a depth of 9 inches (22.9 cm). A minimum of 3 months is needed, 2 months of which temperatures average on a daily basis above 0°C

- Anaerobic digestion: The process is conducted in the absence of air at residence times ranging from 60 days at 20°C to 15 days at 35°C to 55°C, with a volatile solids reduction of at least 38%.

- Composting: Using the within-vessel, static aerated pile or windrow composting methods, the solid waste is maintained at minimum operating conditions of 40°C for 5 days. For 4 hours during this period the temperature exceeds 55°C.

- Lime stabilization: Sufficient lime is added to produce a pH of 12 after 2 hours of contact.

- Techniques demonstrated to be the equivalent of the above on the basis of pathogen and volatile solid reduction.

Once processed sludge is applied to the land, access of the general public to the land-application area must be controlled for 12 months, and grazing of domestic animals on the land must not be allowed for at least 1 month. These access controls apply also to land on which septic-tank pumpings, or septage, is applied. (No prior processing is required of the septic-tank pumpings if control of access is practiced.) If the land is to be used within 18 months for cultivating human food crops, and if the edible portion of the crop is likely to be exposed to the sludge, then either sewage sludge or septic-tank pumpings must be treated by one of the following "Processes to Further Reduce Pathogens" prior to application to the land:

- High-temperature composting
- Heat drying
- Heat treatment
- Thermophilic aerobic digestion
- Irradiation

The processes and their required operating parameters are defined in Appendix II to the Part 257 Criteria.

PURPOSE OF LITERATURE REVIEW

EPA commissioned this literature review in order to bring together in a single document all the available data relevant to 40 CFR Part 257. The completed review comprises:

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- An introductory section, which describes the purpose and scope of the review, and which details the literature search strategy and the rationale used in the review process.
- A summary of the findings of this review, including a comparison of the efficiency of the sludge treatment processes examined.
- Critical review and analysis of technical literature pertaining to densities of selected indicator and pathogenic organisms in raw septage and primary and secondary sludges (Section 3).
- Critical review and analysis of technical literature pertaining to the destruction of selected indicator and pathogenic organisms in sludge subjected to various stabilization and dewatering processes (Sections 4 and 5).
- Presentation of the literature cited in this review with reference to the analytical methodology used (Section 6).
- Compilation of a bibliography of literature that might pertain to the review, but that (for reasons specified later in this section) fails to provide useful quantitative information (Appendix A).
- A description of analytical methodologies used to enumerate the organisms studied by each author whose work is cited (Appendix B).

SCOPE OF REVIEW

A comprehensive review was made of pertinent domestic and foreign literature published since 1940 as technical reports or journal articles, containing quantitative information on the areas of investigation. As shown in Appendix A, a total of 370 references were reviewed and included for citation in the course of this review.

The presence and survival of the following organisms were analyzed:

- Indicators -- Total coliform, fecal coliform, and fecal streptococcus bacteria; *Clostridium perfringens* (welchii); bacteriophage
- Pathogenic bcteria -- Salmonellae, Shigellae, *Pseudomonas* sp., *Mycobacterium* spp., *Candida albicans*, *Aspergillus fumigatus*
- Enteric viruses -- Enterovirus and its subgroups (polioviruses, echoviruses, and coxsackieviruses), reovirus, and adenovirus
- Parasites -- *Entamoeba histolytica*, *Ascaris lumbricoides*, *Taenia* spp., *Schistosoma* spp., and others

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The major objective of this investigation involved analysis of the survival of these organisms in sludge processed by certain stabilization techniques defined in 40 CFR 257 (lime stabilization, mesophilic anaerobic digestion, mesophilic aerobic digestion, and mesophilic composting) and by certain dewatering processes (mechanical dewatering/sludge conditioning, sludge drying, and sludge storage/lagooning).

Within the limitations of the data base, we have:

- Established density levels in raw sludge and septage,
- Identified design and operating variables that affect process efficacy,
- Compared results reported for laboratory/pilot-scale studies to those of full-scale plants,
- Contrasted survival of indicator organisms to that of pathogens, and
- Highlighted areas that require additional directed research.

APPROACH

Literature Search Methodology

In order to assure a complete survey of all literature from 1940 to the present, a number of approaches to accessing the literature were used.

The primary search was conducted using three sources: (1) a computerized search of bibliographic data bases covering literature published from approximately 1970 to the present; (2) a manual review of abstract compendia and literature reviews covering literature produced between 1940 and 1970; and (3) review of approximately 1,000 abstracts compiled by the BDM Corporation, Albuquerque, New Mexico, as part of a pathogen risk analysis program for EPA's Health Effects Research Laboratory.

The computerized search used six data bases. The data bases searched were:

- Compendex: A computer version of Engineering Index covering 3,500 journals, publications of engineering societies, papers from proceedings and government reports from 1970 to the present.
- Biosis Previews: Contains citations from Biological Abstracts and Bioresearch Index covering 8,000 journals, etc., from 1969 to the present.
- NTIS (National Technical Information Service): References 641,000 citations of Government-sponsored research, development, and engineering projects from 1964 to the present.

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- o **Pollution Abstracts**: Contains approximately 63,000 citations on pollution, its sources and control from 1970 to the present.

- o **Sci Search** (Institute for Scientific Information): Accesses about 2,600 major scientific journals worldwide and includes Science Citation Index and Current Contents publications. This is a multidisciplinary data base covering both scientific and technical literature from 1974 to the present.

- o **SSIE Current Research** (Smithsonian Science Information Exchange): Contains approximately 170,000 citations of reports on both government and privately funded scientific and technical research projects initiated or completed during the last three years.

Key words or topics of varying levels of specificity, depending on the data base, were used to scan for relevant citations. The specificity ranged from a crossing of sewage bacteriology AND sludge for the Compendex data base to a listing of 21 genus names AND sludge in Sci Search and Biosis Previews. About 700 citations, some with abstracts, were obtained from the computerized search.

In order to obtain literature published between 1940 and 1970, abstract compendia and literature reviews were searched manually. Topics similar to those used in the computerized search of recent literature were used. The following indexes and literature reviews were examined:

- o Water Pollution Abstracts (Great Britian)

- o Chemical Abstracts

- o Engineering Index

- o Journal of Water Pollution Control Federation
  Annual Literature Review

- o Biosis

In the initial review, all citations and abstracts that dealt with bacteria, viruses, and parasites in sludge and septage were chosen (those in which discussion was limited to sewage and sewage-treatment processes were rejected, unless there was direct mention of sludge or septage). Only those publications that discussed the selected sludge-treatment processes or that enumerated organisms in raw sludge and septage were actually retained for detailed review, however.

Articles and reports were obtained from the CDM in-house library; from various university libraries in the Boston, Massachusetts area; from the Environmental Protection Agency Region I Library; from Gerald Stern, Project Officer, Office of Research and Development; from the Library of Congress, Washington, D.C.; and from individual authors or companies or districts that were conducting pertinent studies.

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Once articles were acquired, a cross-check of important literature cited by authors with the citations obtained from the computer and manual search provided a secondary means of identifying all pertinent citations.

The citations were retained in a coded (by topic) key-sort filing system that allowed cross-referencing of organism with sludge type or treatment process.

Citations of pertinent microbiological articles or reports that were encountered during the literature search but were not utilized in this review are included--organized into nine general categories--in Appendix A. The nine categories of "rejected" literature follow:

(1) Land application. This category encompasses citations of articles or reports that dealt with the application of sludge to land.

(2) Disinfection. Citations of articles that reported on the efficacy of thermoradiation, irradiation, and pasteurization in reducing levels of microorganisms are included in this section.

(3) Thermophilic processes. Articles or reports in this category discuss treatment processes that were conducted in the thermophilic range, including composting at greater than 55°C for more than 4 hours, and thermophilic aerobic digestion.

(4) Other sludge treatment processes. This category encompasses a variety of sludge treatment processes: heat drying, treatment with halogen compounds, ocean disposal, landfilling, entrenchment, and the Carver Greenfield drying process.

(5) Other sludges. Non-conventional sludges are included in this section. Studies that utilized sludges from dairy factories and a chemical sludge from a tertiary treatment plant were encountered.

(6) Other organisms. Included in this section are citations of articles or studies that enumerated (1) organisms that are neither pathogenic nor parasitic and (2) viruses with non-human hosts. Generally, these articles examine the organisms that constitute the normal bacterial population found in sludge treatment process--that is, the organisms whose metabolic activity forms the basis of the sludge treatment process.

(7) Non-quantitative data. All articles and reports included in this section were actually obtained and reviewed. They were not included in the body of this report for one of the following reasons:

- The methodology used to sample or enumerate organisms was not truly quantifiable. For example, Kelly and Sanderson (1960) sampled sludge using absorbent swabs and reported their results as per 100 ml of swab expression. This denominator has no direct relationship to actual densities.

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- o Articles or, most commonly, abstracts of papers presented at meetings, in which authors reported or discussed results but gave no actual data.

- o Only the presence or absence of organisms was noted.

- o Density data in raw sludge was reported on a per ml or on a per gram wet weight basis with no percentage of total solds given to allow conversion to a dry weight basis. This criterion was followed for literature used in Section 3, in order to enable valid comparisons among different authors' findings, and for literature used in discussion of dewatering and drying processes.

(8) Review articles. These articles were obtained and reviewed. They were not, however, included in the body of this report because they did not contain original data.

(9) Articles not obtained. In some cases, citations were obtained from abstract compendia or were referenced by BDM Corporation, but were not cited by other researchers in the field and apparently were not available in final published form.

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SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

A critical review of the literature obtained in this study revealed the dearth of reliable data available on the density levels of indicator and pathogenic organisms, enteroviruses, and parasites in raw sludge and septage.

Similarly, consistent data are lacking relative to the die-off rate of organisms during the sludge stabilization and dewatering processes defined by EPA as "Processes to Significantly Reduce Pathogens". Few of the studies reviewed reported the experimental or operating conditions under which their data were collected. Lack of this information severely hampered the thorough evaluation of the ability of these processes to reduce pathogens and other organisms.

Two general recommendations have resulted from this work. The first is that additional research should be done on the effectiveness of all the treatment processes in reducing levels of organisms. Second, researchers should document all pertinent aspects of their experimental design. Such documentation would provide the foundation for a data base that specifically addresses the reduction of indicator and pathogenic organisms, parasites and viruses.

Of the processes for significantly reducing pathogens, anaerobic digestion and lime stabilization consistently produce reliable reductions of bacterial and (for anaerobic digestion) viral levels.

Anaerobic digestion produces average 1-log to 2-log reductions of indicator bacteria, *Salmonella* and indigenous enterovirus. Parasite levels are not effectively reduced during digestion.

Lime stabilization can produce a greater than 2-log reduction (or result in density levels below the analytical limits of detection) of indicator bacteria, *Salmonella* spp., and *Pseudomonas aeruginosa*, provided that a pH of 12 is maintained for the requisite 2 hours.

An insufficient data base exists for evaluating the effectiveness of aerobic digestion in reducing pathogens. General indications are that the variable responses of bacteria to the digester environment cannot guarantee even a 1-log reduction during this process.

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Mesophilic composting may be an effective means of inactivating total and fecal coliform bacteria, *Salmonella* sp., and viruses, provided that the temperatures specified in 40 CFR Part 257 are maintained throughout the compost pile. The outer "toe" of the pile can be used as a conservative indicator of pile temperature. Responses of the pathogen *Aspergillus fumigatus* and ova of the parasitic roundworm *Ascaris lumbricoides* indicate that these organisms remain a potential problem in sludge treated by mesophilic composting.

The data available do not allow evaluation of air drying of sludge with regard to the criteria specified in 40 CFR Part 257. The research conducted does, however, focus attention on the solids level achieved during drying. This parameter could be a useful criterion for defining air drying of sludge, in addition to the parameters of time, temperature, and sludge depth.

Storage (lagooning) times ranging from 1 month (for bacteria) to greater than 6 months (for parasitic ova) at temperatures greater than 20° C; and from 6 months (for bacteria) to at least 3 years (for parasitic ova) at temperatures of less than 20°C are required to achieve 1-log reduction of the organisms studied.

The process of mechanical dewatering of municipal wastewater sludges alone has little effect on the density levels of pathogens. Three conditioners are commonly used in combination with mechanical dewatering: polymer, ferric chloride ($FeCl_3$), and lime. Polymer has no apparent effect on density levels of pathogens. $FeCl_3$, often used in conjunction with lime, appears to reduce the virucidal and bactericidal effects of this conditioner. Lime, added in concentrations to optimize dewatering, cannot be relied on to reduce pathogen levels because of the variations in pH levels obtained.

Few of the laboratory-scale studies reviewed could be related to results obtained at full-scale treatment plants. Operating parameters used in laboratory experiments differed radically from those at full-scale plants. For this reason, comparing density levels was seldom possible. In addition, laboratory studies often used seeded bacteria, viruses, or parasites. It is questionable whether their behavior mimics that of naturally occurring organisms.

No single indicator organism (either bacteria or bacteriophage) was found to maintain a density level of a constant relative value to that of pathogenic organisms. The data available made it impossible to determine whether this inconsistency is due to the inability of current techniques to enumerate pathogenic bacteria and enteroviruses accurately, or to the fact that densities actually vary.

Of the traditional indicators, fecal streptococci appear to be the most conservative indicator of both the density levels of pathogenic bacteria and enterovirus in raw sludge and of their reduction during sludge treat-

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ment processes. Additional research is required to identify other indicator systems, both bacterial and viral, whose numbers better reflect both density and reduction of pathogenic organisms.

A wide variety of methods were used to enumerate all of the organisms considered in this review. Although *Standard Methods* are available for quantifying the coliform and streptococcus bacteria, and for *Salmonella* sp., there are no standard techniques for other pathogens, enteroviruses, or parasites. It is recommended that this area be addressed so that comparable data can be produced in future studies.

SECTION 3

DENSITY LEVELS OF INDICATOR AND PATHOGENIC ORGANISMS IN RAW MUNICIPAL WASTEWATER SLUDGES AND SEPTAGE

This section presents an overview of available data on the densities of indicator and pathogenic organisms and parasites in raw sludge and septage. Density levels are expressed as the number of organisms per gram dry weight (no./GDW). Where information was available only as number of organisms per 100 ml, conversions to no./GDW were made if the sludge total solids (TS) concentration were known.

In the tables of data that follow, geometric means have been developed for each organism from data presented by each researcher or research team. Recognizing that wide variation exists among the densities reported--especially for pathogenic organisms, as would be expected from their low densities and patchy distribution in sludge--we have nevertheless computed average geometric means for each organism in each type of sludge.

PRIMARY SLUDGE

Density levels of indicator, pathogens, viruses, and parasites in primary sludge are presented in Table 1.

Average (of geometric means) counts of 1.2×10^8 total coliform bacteria per gram dry weight (GDW), 2.0×10^7 fecal coliform bacteria and 8.9×10^5 fecal streptococci were calculated from data from four treatment plants.

Bacteriophage--enumerated by two research teams--Nath and Johnson (1979) and Moore *et al.* (1978)--occur at densities of 1.3×10^5 plaque forming units (PFU) per GDW, with a range of 10^3 to 10^6. The use of bacteriophage as indicators of the density and survival of enteric virus has been studied in water and wastewater systems, primarily because of the ease of enumerating bacteriophage compared to eucaryote viruses. Nath and Johnson (1979) computed the ratio of coliphage to enteroviruses in the primary sludge to determine if a constant relative value existed. They found that the ratio varied from a maximum of 28,000:1 to a minimum of 13:1, indicating that no relationship exists between the numbers of the two types.

Two pathogenic bacteria, *Salmonella* sp. and the secondary pathogen *Pseudomonas aeruginosa* occur in primary sludge at typically lower concentrations than indicator bacteria. An average *Salmonella* density of 4.1×10^2 per GDW and a *Ps. aeruginosa* density of 2.8×10^3 per GDW were calculated.

TABLE 1. LEVEL OF INDICATOR AND PATHOGENIC ORGANISMS IN RAW PRIMARY SLUDGE

	Densities of bacteria, viruses and Ascaris ova (organisms per gram dry weight)									
Organism	Total Coliforms	Fecal Coliforms	Fecal Streptococci	Bacteriophage[a]	Salmonella	Pseudomonas aeruginosa	Enteric Virus	Ascaris sp. Ova	Location/Sludge Characteristics	Reference
Geometric Mean N[b] Range	1x10^8 8 NS[c]	1x10^8 8 NS	1x10^4 8 NS						Johnson City WWTP, TN (5% TS)	Gaby, 1975
Geometric Mean N Range				8.9x10^4 PFU 7 4.3x10^3 to 8x10^6			1.7x10^2 PFU[d] 14 1.1x10^2 to 2.5x10^3		James River WWTP, VA (0.9-4.7% TS)	Nath and Johnson, 1979
Geometric Mean N Range				9.8x10^4 NA[e] NA			4.4x10^2 PFU NA NA		Butte, MT	Moore *et al.*, 1978
Geometric Mean N Range					5.3x10^5 PFU 3 3.4x10^3 to 2.1x10^5		2.2x10^3 PFU 3 7.9x10^2 to 4.3x10^3		Boulder, CO	Moore *et al.*, 1978
Geometric Mean N Range		6.7x10^6 MPN 6 8.4x10^5 to 2.1x10^7	3.7x10^6 MPN 6 2.2x10^6 to 4.8x10^6		1.4x10^3 MPN 6 9.7x10^7 to 2.0x10^3	6.9x10^3 MPN 4 2.0x10^3 to 3.1x10^4			Richland, WA	Counts and Shuckrow, 1974

[a] Host cell: *Escherichia coli*
[b] N = Number of samples
[c] NS = Not specified by author
[d] Geometric mean of virus enumerated on BGM and VERO cells, PFU = Plaque Forming Units
[e] NA = Not applicable, only single analysis performed
[f] ND = Not detected

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TABLE 1 (continued)

Organism	Densities of bateria, viruses and Ascaris ova (organisms per gram dry weight)									
	Total Coliforms	Fecal Coliforms	Fecal Streptococci	Bacteriophage[a]	*Salmonella*	*Pseudomonas aeruginosa*	Enteric Virus	Parasite Ova/Cysts	Location/Sludge Characteristics	Reference
Geometric Mean N Range	5.9x10^{8} MPN NS 2.7x10^{8} to 7x10^{8}	1.7x10^{8} MPN NS 2.7x10^{7} to 6.8x10^{8}	8.0x10^{6} MPN NS 5.4x10^{6} to 1.1x10^{7}		1.27x10^{1} MPN NS 2.3 to 4.93x10^{1}	4.0x10^{1} NS 1.54x10^{1} to 9.1x10^{1}			Lebanon, OH	Noland *et al.*, 1978
Geometric Mean N Range		2.8x10^{6} 6 NS	1.5x10^{6} 6 NS		5x10^{0} 6 NS				Sacramento Regional Treatment Plant, 1977	Sacramento Area Consultants, 1979
Geometric Mean N Range		3.0x10^{7} 6 NS	3.5x10^{7} 6 NS		ND[f] 6 NS				Sacramento Regional Treatment Plant, 1976	Sacramento Area Consultants, 1979
Geometric Mean N Range								1.9 ova 15 ND - 105	Tampa Municipal Sewage Disposal Plant, FL (4.7-5.5% TS)	Bond, 1958
Geometric Mean N Range								1.4x10^{3} ova 4 8.3x10^{2} to	Cyda, S.W. Johannesburg, South Africa (1.9-5.2% TS)	Keller and Hide, 1951
Geometric Mean N Range								1.3x10^{3} ova 4 9.8x10^{2} to	Bruma, S.W. Johannesburg, South Africa (3.8-6.4% TS)	Keller and Hide, 1951

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TABLE 1 (continued)

Densities of bacteria, viruses and *Ascaris* ova (organisms per gram dry weight)

Organism	Total Coliforms	Fecal Coliforms	Fecal Streptococci	Bacteriophage[a]	*Salmonella*	*Pseudomonas aeruginosa*	Enteric Virus	Parasite Ova/Cysts	Location/Sludge Characteristics	Reference
Geometric Mean N Range								3.8×10^3 3 2.6×10^3 7.8×10^3	Klipspruit, S.W. Johannesburg, South Africa (3.6-9.9% TS)	Keller and Hide, 1951
Geometric Mean N Range								4.0×10^1 NA NA	Cape Town City, South Africa	Steer *et al.*, 1974
Geometric Mean N Range								5.0×10^1 NA NA	Macon, GA	Theis *et al.*, 1978
Geometric Mean N Range							5.9×10^0 3 NS		Houston, TX	Hurst *et al.*, 1978
Geometric Mean N Range							1.9×10^3 TCID$_{50}$ 4 4×10^2 to 9×10^3		Damhusaaen, Denmark (2.9-5.5% TS)	Mielson and Lydholm, 1979
AVERAGE GEOMETRIC MEAN	1.2×10^8	2.0×10^7	8.9×10^5	1.3×10^5 PFU	4.1×10^2	2.8×10^3	3.9×10^2 PFU	2.1×10^2 ova		

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TABLE 1 (continued)

Densities of viable parasite ova (ova per kg dry weight). All samples collected over four seasons from 17 WWTPs in southern U.S. Reference: Reimers *et al.* (1980)

Organism	*Ascaris* sp.	*Trichiuris trichiura*	*Trichiuris vulpis*	*Toxocara* sp.	*Hymenolepis diminuta*
Geometric Mean N Range	9,000 4 7,100-10,000	110 4 0-1,800	180 4 0-1,500	1,900 4 800-3,300	20 4 0-1,200
Geometric Mean N Range	19,200 4 7,800-32,000	0 4 0	120 4 0-960	2,500 4 1,600-4,000	260 4 0-2,800
Geometric Mean N Range	1,800 4 830-3,600	0 4 0	590 4 310-1,400	220 4 0-3,900	20 4 0-340
Geometric Mean N Range	1,200 4 580-2,700	<10 4 0-190	480 4 0-580	190 4 0-2,700	<10 4 0-910
Geometric Mean N Range	<10 3 0-210	0 3 0	40 3 0-220	1,200 3 650-2,400	0 3 0
Geometric Mean N Range	1,700 4 640-4,500	130 4 0-760	60 4 0-3,600	<10 4 0-2,000	<10 4 0-650
Geometric Mean N Range	<10 4 0-580	0 4 0	310 4 190-480	770 4 0-1,200	0 4 0
Geometric Mean N Range	90 4 0-900	0 4 0	10 4 0-300	460 4 270-1,200	<10 4 0-480

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TABLE 1 (continued)

Densities of viable parasite ova (ova per kg dry weight). All samples collected over four seasons from 17 WWTPs in southern U.S. Reference: Reimers *et al.* (1980)

Organism	*Ascaris* sp.	*Trichiuris trichiura*	*Trichiuris vulpis*	*Toxocara* sp.	*Hymenolepis diminuta*
Geometric Mean N Range	90 4 0-890	0 4 0	0 4 0	580 4 260-1,000	0 4 0
Geometric Mean N Range	1,400 4 660-4,500	90 4 0-600	120 4 0-1,700	850 4 240-1,500	<10 4 0-880
Geometric Mean N Range	11,800 4 9,300-16,000	8,200 4 4,800-16,000	160 4 90-290	650 4 200-1,900	0 4 0
Geometric Mean N Range	20 4 0-310	0 4 0	20 4 0-630	90 4 0-850	0 4 0
Geometric Mean N Range	130 3 0-1,700	0 3 0	350 3 40-1,200	90 4 0-860	0 4 0
Geometric Mean N Range	470 3 210-1,000	0 3 0	2,700 3 2,200-3,900	140 3 0-1,700	<10 3 0-240
Geometric Mean N Range	7,500 4 3,800-12,000	0 4 0	20 4 0-330	70 4 0-660	40 4 0-5,000
Geometric Mean N Range	16,300 3 12,000-19,000	7,700 3 6,000-10,000	1,200 3 0-1,900	640 3 230-2,000	<10 3 0-160
Geometric Mean N Range	4,500 4 1,200-9,700	440 4 0-6,200	1,200 4 890-1,700	130 4 0-2,700	30 4 0-1,800
TOTAL Geometric Mean N Range	720 64 0-32,000	10 64 0-16,000	110 64 0-3,900	240 64 0-3,900	6 64 0-5,500

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Enteric virus in samples from five wastewater treatment plant averaged 4 x 10^2 PFU per GDW.

Density levels of parasite ova in raw primary sludge are presented in Table 1. Most data were obtained from a study conducted by Reimers *et al.* (1980) in which 27 municipal WWTPs were sampled quarterly over a one-year period. At 17 of the plants, primary sludge was sampled and examined for parasite ova.

Reimers *et al.* reports that ova of *Ascaris* sp. were found in the greatest density, a mean of 720 ova per kg dry weight (0.72 per GDW). This was said to be at least partially due to the higher fertility rate of *Ascaris* compared to other parasites. Other authors have found a range of 2 to 50 ova of *A. lumbricoides* per GDW (Theis *et al.*, 1978, and Bond, 1958) in U.S. treatment plant sludges, while *Ascaris* levels in primary sludge from Johannesburg, South Africa contained up to 7,800 ova per GDW. In the Reimers *et al.* study, *Trichiuris trichiura* (the human whipworm) occured at a mean density of 10 per kg DW; *T. vulpis* (the dog whipworm) at a mean density of 110 per kg DW; and *Toxocara* sp. (most likely *T. canis*, the dog roundworm) at a mean density of 240 per kg DW. The comparatively frequent occurrence of *Hymenolepis diminuta* (geometric mean of 6 per kg DW), the rat tapeworm, is an indication of the residency of rats in and around treatment plants.

Entamoeba coli-like cysts were the protozoan cysts found most commonly by Reimer *et al.* in all sludges. Viable cysts were found in only one sample, however. Since the cysts of *E. coli* are more resistant than those of *E. histolytica*, the human pathogen, it was thought unlikely that their ova, if present, could pass through in a viable state. *Giardia* cysts were found in samples, but none appeared viable.

SECONDARY SLUDGE

Levels of indicator and pathogenic bacteria, enteroviruses and parasites are presented in Table 2. In spite of the smaller data base, it would appear that concentrations are similar to those reported in raw primary sludge.

Total coliform bacteria occur at average densities of 7 x 10^8 per GDW, fecal coliforms at 8.3 x 10^6, and fecal streptocccci at 1.7 x 10^6.

Salmonella sp. averaged 8.8 x 10^2 and *Ps. aeruginosa*, 1.1 x 10^4 per GDW. Enterovirus densities from three treatment plants ranged from 1.7 x 10^2 to 2.2 x 10^3 PFU per GDW (Nath and Johnson, 1979, and Moore *et al.*, 1978). Bitton *et al.* (1980) reports that enterovirus levels in two samples of waste activated sludge ranged from 11 to 25 50-percent tissue culture infective dose ($TCID_{50}$). Data on density levels of parasite ova in secondary sludge were obtained from Reimers *et al.* (1980). *Ascaris* in sludges from five WWTPs averaged 1,360 per kg DW; *T. trichiura* averaged less than 10 per kg DW; *T. vulpis*, less than 10 per kg DW; *Toxocara* sp., 280 per kg DW; and *H. diminuta*, 20 per kg DW.

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TABLE 2. LEVELS OF INDICATORS AND PATHOGENS IN RAW SECONDARY SLUDGE

	Densities of bacteria and viruses (organims per gram dry weight)							
Organism	Total Coliforms	Fecal Coliforms	Fecal Streptococci	Salmonella	Pseudomonas aeruginosa	Enteric Virus	Location/Sludge Characteristics	Reference
Geometric Mean N[c] Range		6.63×10^6 MPN 6 1.1×10^6 to 1.9×10^7	2.1×10^6 MPN 6 1.4×10^6 to 2.9×10^6	2.1×10^3 6 7.4×10^2 to 5.8×10^3	1.2×10^4 6 1.6×10^3 9.4×10^4		Richland, WA Trickling filter	Counts and Shuckrow, 1974
Geometric Mean N Range	6.7×10^8 NS[a] 1.4×10^8 to 1.2×10^9	2×10^7 NS 1.6×10^7 to 2.7×10^7	8.4×10^6 NS 4.1×10^5 to 1.6×10^7	4.9×10^0 NS 2.4×10^0 to 7.3×10^0	4.5×10^3 NS 7.4×10^2 to 8.9×10^3		Lebanon, OH Waste activated	Noland *et al.*, 1978
Geometric Mean N Range						3.84 PFU NA[b] NA	Houston, TX Waste Activated	Hurst *et al.*, 1978
Geometric Mean N Range						2.9×10^2 PFU 2 1.8×10^2 to 4.6×10^2	Butte, MT Waste Activated	Moore *et al.*, 1978
Geometric Mean N Range						3.4×10^2 3 2.7×10^2 to 5.3×10^2	Austin, TX Waste Activated	Moore *et al.*, 1978

[a] NS = Not specified by author

[b] NA = Not applicable, only single analysis performed

[c] N = Number of samples

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Organism	Total Coliforms	Fecal Coliforms	Fecal Streptococci	*Salmonella*	*Pseudomonas aeruginosa*	Enteric Virus	Location/Sludge Characteristics	Reference
	Densities bacteria and viruses (organisms per gram dry weight)							
Geometric Mean N Range						2.5×10^1 $TCID_{50}$ NA NA	Gainesville, FLA Waste activated (12/77)	Bitton *et al.*, 1980
Geometric Mean N Range						1.1×10^1 $TCID_{50}$ NA NA	Gainesville, FLA Waste activated (2/78)	Bitton *et al.*, 1980
Geometric Mean N Range	7.1×10^8 5 4.7×10^8 to 1.0×10^9	9.1×10^6 5 6.1×10^6 1.3×10^7	8.9×10^6 5 2.9×10^6 1.6×10^7				Pullman, WA Waste Activated (3.1-6.2% TS)	Bovay Engineering, 1975
AVERAGE GEOMETRIC MEAN	7.0×10^8	8.3×10^6	1.7×10^6	8.8×10^2	1.1×10^4	3.2×10^2 PFU		

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Densities of viable parasite ova (ova per kg dry weight). Samples of raw, settled activated sludge were collected in the fall (3 plants), fall and winter (1 plant), and in all four seasons (1 plant). Reference: Reimers *et al.* (1980)

Organism	*Ascaris* sp.	*Trichiuris trichiura*	*Trichiuris vulpis*	*Toxocara* sp.	*Hymenolepis diminuta*
Geometric Mean N Range	4,100 2 3,900-4,300	0 2 0	0 2 0	70 2 0-4,700	30 2 0-780
Geometric Mean N Range	0 1 -	0 1 -	0 1 -	1,400 1 -	0 1 -
Geometric Mean N Range	10,000 1 -	7,700 1 -	1,500 1 -	440 1 -	150 1 -
Geometric Mean N Range	2,800 1 -	0 1 -	0 1 -	900 1 -	940 1 -
Geometric Mean N Range	2,400 4 1,400-3,300	<10 4 0-790	<10 4 0-760	250 4 0-4,000	<10 4 0-1,000
TOTAL Geometric Mean N Range	1,360 9 0-10,000	<10 9 0-7,700	<10 9 0-1,500	280 9 0-4,700	20 9 0-1,000

MIXED SLUDGE

A great deal of data exists for the density levels of indicators, pathogens and parasites in mixed raw sludge. However, very few data were found on enteric viruses in mixed raw sludge. The data for these organisms are presented in Table 3.

Levels of total coliform bacteria, obtained from data from only one treatment plant (Berg and Berman, 1980) are 1.1×10^9 per GDW. Fecal coliform bacteria and fecal streptococci averaged 1.9×10^5 and 3.7×10^6 per GDW. It should be noted that much of the data used in these averages were gathered from a study by LaConde (1978). Levels of both organisms are lower than would be predicted based upon their densities in primary and secondary sludge. The sample preparation technique used by this team prior to enumeration of bacteria was a washing technique where sterile water was used to elute bacteria and solids were discarded. This procedure would lose any bacteria that were associated with solids.

Levels of *Salmonella* sp. and *Shigella* sp. reported by LaConde, (1978) were generally below analytical detection limits. These results can again be attributed to the sample preparation technique used. Counts and Shuckrow (1974) reported *Salmonella* sp. at densities of 10^2 to 10^3 per GDW.

Ps. aeruginosa, also enumerated by these authors, averaged 3.3×10^2 per GDW.

Enteric virus from two treatment plants in Denmark (Nielson and Lydholm, 1979) average 3.6×10^2 $TCID_{50}$.

Total parasite ova in sludges from eight sewage treatment plants ranged from not detected to 50 ova per GDW (LaConde, 1978). *Toxocara canis*, and *Ascaris lumbricoides* were most commonly isolated in these samples. Similarly, *Ascaris* and *Toxocara* sp. were most commonly enumerated in mixed sludges from four southern U.S. WWTPs (Reimers *et al*,, 1980). *Ascaris* occurred at a mean density of 290 per kg DW (0.29 per GDW); *Toxocara* sp. at 1,300 per kg DW (1.3 per GDW) and *Trichiuris vulpis* at 140 per kg DW, (Table 3). In comparison to both primary and secondary sludges also examined in this study, neither *T. trichiura* nor *Hymenolepis diminuta* were isolated from the eight samples examined.

SEPTAGE

Total and fecal coliform and fecal streptococci concentrations found in septage appear to be similar to concentrations found in raw sludge. *Salmonella sp.* and *Pseudomonas aeruginosa* concentrations found in septage appear to be lower than in raw sludge, but no generalizations can be drawn based on such a limited data base. Data are presented in Table 4.

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TABLE 3. LEVELS OF INDICATOR BACTERIA AND PATHOGENIC ORGANISMS IN RAW MIXED SLUDGE

	Densities of bateria, viruses, and parasite ova (organism per gram dry weight)									
Organism	Total Coliforms	Fecal Coliforms	Fecal Streptococci	Salmonella	Shigella	Pseudomonas aeruginosa	Enteric Virus	Parasite Ova/Cysts	Location/Sludge Characteristics	Reference
Geometric Mean N[a] Range	$1.1x10^9$ 11 $4.3x10^7$ to $3.4x10^9$	$6.6x10^7$ 11 $1.4x10^6$ to $2.5x10^8$	$6.0x10^6$ 11 $3.7x10^5$ to $1.4x10^7$						Los Angeles Hyperion Treatment Plant, CA 2/3 primary + 1/3 waste activated (3.5% TS)	Berg and Berman, 1980
Geometric Mean N Range		$3.1x10^1$ MPN 2 ND[b]-$9.3x10^2$	$5.0x10^6$ 2 $8.0x10^4$ to $3.1x10^8$	ND 1 NA[c]	ND 2 NA			ND 2 NA	Las Virgenes, CA Primary + waste activated (3% TS)	LaConde, 1978
Geometric Mean N Range		$8.3x10^1$ MPN 2 $5.6x10^1$ to $1.2x10^2$	$1.7x10^6$ 2 $1.9x10^4$ to $1.5x10^8$	ND 2 NA	ND 2 NA			ND 2 NA	Macon, GA Primary + trickling filter	LaConde, 1978
Geometric Mean N Range		$5.7x10^2$ MPN 2 $3x10^2$ to $1.0x10^3$	$7.6x10^6$ MPN 2 $1.2x10^5$ to $4.8x10^8$	ND 2 NA	ND 2 NA			<50 ova 2 NA	Springfield, MO Primary + trickling filter	LaConde, 1978
Geometric Mean N Range		$1.1x10^4$ MPN 2 $3.0x10^3$ to $3.8x10^4$	$2.5x10^7$ 2 $4.0x10^6$ to $1.6x10^8$	$4.2x10^{-1}$ 2 0 to $8.3x10^{-1}$	ND 2 NA			<50 ova 2 NA	Hopkinsville, KY Primary + trickling filter	LaConde, 1978

[a] N = Number of samples
[b] ND = Not detected
[c] NA = Data not applicable per gram total suspended solids
[d] = per gram total suspended solids

(continued)

TABLE 3. (continued)

Organism	Densities of bacteria, viruses, and parasite ova (Organism per gram dry weight)									
	Total Coliforms	Fecal Coliforms	Fecal Streptococci	Salmonella	Shigella	Pseudomonas aeruginosa	Enteric Virus	Parasite Ova/Cysts	Location/Sludge Characteristics	Reference
Geometric Mean N Range	1.1x10^1 MPN	1.1x10^1 MPN 2 0 to 1.1x10^2	8.5x10^6 MPN 2 1.5x10^5 to 4.8x10^8	ND 2 NA	ND 2 NA			50 ova 2 NA	Frankfort, IN Primary + trickling filter	LaConde, 1978
Geometric Mean N Range		3x10^3 MPN 2 5.6x10^2 to 1.6x10^4	2.4x10^7 2 1.6x10^6 3.5x10^8	ND 2 NA	ND 2 NA			ND 2 NA	Wilmington, OH Activated sludge + primary	LaConde, 1978
Geometric Mean N Range		1.8x10^2 MPN 2 0 to 3.1x10^4	5.4x10^6 MPN 2 2.4x10^6 to 1.2x10^7	ND 2 NA	ND 2 NA			ND 2 NA	Kendallville, IN Primary + trickling filter	LaConde, 1978
Geometric Mean N Range		5.0x10^4 2 3.2x10^3 to 7.7x10^5	1.1x10^5 2 1.6x10^3 to 8.0x10^7	ND 2 NA	ND 2 NA			ND 2 NA	Columbus, IN Primary + waste activated	LaConde, 1978
Geometric Mean N Range		8.8x10^6 2 8.2x10^6 to 9.5x10^6	3.3x10^6 2 3.2x10^6 to 3.4x10^6	1.8x10^2 2 1.4x10^2 to 2.3x10^2		2.0x10^2 2 1.6x10^2 to 2.6x10^2			Richland, WA Primary + trickling filter (2% TS)	Counts and Shuckrow, 1974

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TABLE 3. (continued)

	Densities (Organism per gram dry weight)									
Organism	Total Coliforms	Fecal Coliforms	Fecal Streptococci	Salmonella	Shigella	Pseudomonas aeruginosa	Enteric Virus	Parasite Ova/Cysts	Location/Sludge Characteristics	Reference
Geometric Mean N Range		4.1×10^6 2 3.6×10^6 to 4.6×10^6	9.9×10^6 2 9.4×10^6 to 1.1×10^7	1.8×10^3 2 1.3×10^3 to 2.5×10^3		1.2×10^3 2 6.4×10^2 to 1.9×10^3			Richland, WA Primary + Trickling filter (4.4% TS)	Counts and Shuckrow, 1974
Geometric Mean N Range		8.5×10^6 6 3.1×10^6 to 1.9×10^7	2.4×10^6 6 1.5×10^6 to 4.6×10^6	1.6×10^3 6 1.2×10^3 to 2.2×10^3		1.2×10^4 6 6.5×10^3 to 2.0×10^4			Richland, WA Primary + trickling filter (3-5% TS)	Counts and Shuckrow, 1974
Geometric Mean N Range							4.8×10^2 $TCID_{50}$[d] 2 1.0×10^2 to 2.3×10^3		Nivaa, Denmark Biological filter, Activated + primary sludge (3.0% TS)	Nielson and Lydholm, 1979
Geometric Mean N Range							2.8×10^2 $TCID_{50}$[d] 2 2.0×10^2 to 4.0×10^2		Roskilde, Denmark Biol. filter, Activated + primary sludge (2.1% TS)	Nielson and Lydholm, 1979
AVERAGE GEOMETRIC MEAN	1.1×10^9	1.9×10^5	3.7×10^6	2.9×10^2	ND	3.3×10^3	3.6×10^2 $TCID_{50}$	<50 ova		

(continued)

TABLE 3 (continued)

Densities of viable parasite ova in raw mixed sludge (ova per kg dry weight). Reference: Reimers *et al.* (1980)

Organism	*Ascaris* sp.	*Trichiuris trichiura*	*Trichiuris vulpis*	*Toxocara* sp.	*Hymenolepis diminuta*	Sludge Characteristics
Geometric Mean N Range	12,400 2 10,000-14,000	0 2 0	0 2 -	520 2 270-990	0 2 -	Primary and activated sludge
Geometric Mean N Range	90 3 0-1,100	0 3 -	940 3 540-1,400	1,800 3 1,400-2,700	0 3 -	Primary and activated sludge
Geometric Mean N Range	20 2 0-340	0 2 -	710 2 670-750	3,800 2 3,000-4,700	0 2 -	Primary and contact stabilized sludge
Geometric Mean N Range	1,300 1 -	0 1 -	420 1 -	420 1 -	0 1 -	Primary and activated sludge
TOTAL Geometric Mean N Range	290 8 0-14,000	0 8 -	140 8 1,400	1,300 8 0-4,700	0 8 -	

TABLE 4. LEVELS OF INDICATORS AND PATHOGENS IN RAW SEPTAGE

Densities of bacteria, viruses, and parasite ova (organism per gram dry weight)

Organism	Total Coliforms	Fecal Coliforms	Fecal Streptococci	*Salmonella*	*Pseudomonas aeruginosa*	Location	Reference
Geometric Mean N[a] Range	1.4×10^8 NS[b] 8.5×10^6 - 3.3×10^8	7.1×10^6 NS 4.7×10^6 - 8.5×10^6	3.2×10^5 NS 1.6×10^5 - 5.7×10^5	2.8×10^0 NS 1.4×10^0 - 4.3×10^0	3.5×10^2 NS 7.0×10^2 - 9.9×10^2	Lebanon, OH	Noland *et al.*, 1978
Geometric Mean N Range		6.7×10^5 MPN 4 2.4×10^5 - 1.9×10^6	7.5×10^5 MPN 4 1.1×10^5 - 6.9×10^6	9.4×10^{-2} NA[c] NA	1.9×10^0 NA NA	Lebanon, OH (4-5% TS)	Feige *et al.*, 1975
AVERAGE GEOMETRIC MEAN	1.4×10^8	1.2×10^6	6.6×10^5	0.51	25.5		

[a] N = Number of samples
[b] NS = Not specified by author
[c] NA = Not applicable, only a single analysis performed

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SECTION 4

CONVENTIONAL SLUDGE STABILIZATION PROCESSES

This section presents a critical review of the literature which report on four sludge stabilization processes: mesophilic anaerobic digestion, aerobic digestion, mesophilic composting, and lime stabilization.

Each stabilization process subsection is organized into Introduction, Results and Discussion subsections. The introductory portion defines the stabilization process and briefly describes each article reviewed (Data Base Available).

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Following the Introduction, the Results section presents log reductions and final densities of organisms. The results are organized into four major categories: indicator organisms, pathogenic organisms, enteric viruses and parasites. When sufficient data are available, these categories are further divided by the various parameters that may affect density levels and the scale at which studies were conducted.

The Discussion section evaluates the reductions reported, from the viewpoints both of scale and of the operating parameters controlled. If possible, density levels and reductions of indicator organisms are contrasted to those of pathogens and enteroviruses.

ANAEROBIC DIGESTION

Introduction

Description of Process. Anaerobic digestion is the biological degradation of complex organic substances present in wastewater sludges in the absence of free oxygen. Primary or secondary sludge, or a mixture of both, is fed continuously or intermittently into an airtight reactor vessel and retained for varying detention times. Defined by EPA as a process to significantly reduce pathogens, anaerobic digestion must be conducted in the absence of air at residence times ranging from 60 days at 20°C to 15 days at 35-55°C, with a volatile solids reduction of at least 38 percent (40 CFR Part 257).

Operating Parameters. Three basic variations of anaerobic digestion are commonly practiced: low (or standard) rate digestion, high-rate digestion, and a combination of the two, known as the two-stage process.

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In low-rate digestion, the digesters are unmixed. The functions of digestion, sludge thickening and supernate formation are carried out simultaneously. In high-rate digestion, the contents of the digester are heated to either mesophilic (30-38°C) or thermophilic (50-60°C) temperatures and continuously mixed. As the name implies, high-rate digesting produces a stabilized sludge product at shorter detention times than low-rate digestion.

In the two-stage process, a high-rate digester is coupled in series with a second digestion tank that usually is similar in design to a low-rate digester (unheated and unmixed). The primary (high-rate) tank is used for active digestion, and the secondary tank for settling.

Following are some of the parameters that may be varied in operation of digester systems:

a. Detention time -- Detention time usually varies from 30-60 days for low-rate digesters to 10-20 days for high-rate digesters. The anaerobic digestion process is limited primarily by the growth rate of methane-producing bacteria, which is in turn limited by temperature and food supply. Detention time in the reactor is, therefore, strongly affected by the temperature at which the process is conducted.

b. Temperature -- Digesters can be operated at temperatures ranging from 10°C to 60°C. Commonly, operating temperatures are maintained within the mesophilic range (30-38°C), although thermophilic digesting (50-60°C) offers the advantages of lower detention times, improved dewatering of digested sludge, and increased pathogen destruction.

c. pH -- pH is commonly maintained in the optimum range for methanogenic bacteria, pH 6.5-7.5.

d. Volatile solids reduction -- A common measure of digester performance is the percent of volatile solids (VS) destroyed. Reduction usually ranges between 35 to 60 percent, depending on character of the sludge, detention time and temperature.

e. Type of sludge -- Digesters can be operated on raw primary or secondary sludge or on a mixture of both. Septic-tank pumpings (septage) are usually added at the head of the plant and so are not treated as separate feed to digestion units. Since each sludge is unique, the method of its digestion depends on its specific characteristics and on plant design.

Data Base Available. The following is a brief synopsis of articles and reports that were selected for inclusion in this review. This section is divided into three portions: a. and b., covering, respectively, laboratory/pilot-scale studies and full-scale studies that follow the reduction of organisms through anaerobic digestion, and c., studies that present density levels after digestion without indicating reductions of organisms incurred during the process.

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a. Laboratory or pilot-scale studies:

- Bertucci *et al.* (1977). Survival of seeded poliovirus type 1, coxsackie A9 and B1, and echovirus 11 over a 48-hr digestion period were compared in this experiment. Laboratory-scale digesters were operated at a detention time of 14 days at 35°C.

- Cram (1943). Batch digesters that were unmixed and unheated were utilized in this study. Survival of seeded *A. lumbricoides* and *E. histolytica* was followed over a maximum of 200-days' detention.

- Eisenhardt. *et al.* (1977). Reduction of seeded coxsackie B-3 virus was studied during anaerobic digestion. The laboratory-scale digester was stirred; the detention time was 35 days at 35°C.

- Fenters *et al.* (1979). The levels of seeded enteroviruses (polio type 7, echo 6, and coxsackie B4) seeded into anaerobic sludge supernate were monitored at 20°, 25°, 30° and 35°C. In addition, the response of poliovirus type 1 to ammonia-nitrogen concentrations ranging from 0 to 1,500 mg/l was examined.

- Fitzgerald and Ashley (1977). Ova from the uteri of *Ascaris lumbricoides* var *suum* were held in anaerobically digested sludge for 21 days at 38°C. The researchers determined what percent of ova were able to embryonate following this treatment.

- Fitzgerald and Prakasam (1978). Laboratory-scale digesters were fed sludge from the West Southwest plant of the Metropolitan Sanitary District of Greater Chicago. Encysted *Trichinella spiralis* larvae obtained from the diaphragms, tongues, and masseter and skeletal muscles of laboratory rats were seeded into mixed digesters with a 14-day detention time at 35°C.

- Heukelekian and Albanese (1956). Batch digestion on a laboratory scale was conducted for 35 days at an unspecified temperature. Reduction of tubercle bacteria (*Mycobacterium* sp.) seeded into the digesters was monitored.

- Jones *et al*. (1947). Conducted laboratory-scale experiments in which *Schistosoma japonicum* ova obtained from the feces of infected dogs were inoculated into sludge, which was then digested for up to 26 days at 24-29°C.

- Kruse (1977). Reviewed current literature on survival of *A. lumbricoides* during anaerobic digestion.

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- McKinney *et al.* (1958). High-rate, gas-mixed, feed-and-draw (12 and 24 hour cycle) anaerobic digesters were used to examine the survival of indigenous *Escherichia coli* and seeded *Salmonella typhosa*. Detention times used were 6, 8, 10, 15, and 20 days at a temperature of 29°C. VS reduction ranged from 52 to 58 percent. Organisms were enumerated in this study using a non-standard "spot-plate" technique.

- Newton *et al.* (1948). Laboratory-scale experiments were conducted as a continuation of Jones *et al.* experiments. *S. japonicum* were inoculated into sludge that was then digested for up to 10 weeks at 7 to 18°C.

- Rawn and Candell (1950). These investigators examined anaerobic digestion in a four-stage continuous digester with a total detention time of 11 days at 29°C. Samples from the fourth stage were removed, and batch digestion was continued at 29°C for an additional 49 days. Total coliform bacteria were enumerated in this study.

- Reimers *et al.* (1980). Ova of *Ascaris suum* (from the intestinal contents of swine) and *Toxocara canis* were inoculated into laboratory-scale digesters in this study. Digesters were operated at 35°C and 45°C and were fed with a mixture of primary and return activated sludge at a rate to produce a 15-day hydraulic retention time. Ova were enumerated and examined for viability. Sludges that had been anaerobically digested at 27 WWTPs in the southern U.S. were also examined for parasite content.

- Sanders *et al.* (1979). Bench-scale anaerobic digesters utilizing sludge from the Govalle Treatment Plant, Austin, Texas, were operated at detention times of from 5 to 15 days at temperatures of 34°C to 50°C. The survival of seeded poliovirus type 1 (CHAT) was monitored.

- Silverman and Guiver (1960). Batch anaerobic digesters, unmixed and held at 35°C, were used in this experiment. Ova of *Taenia saginata* were suspended in the digesting sludge, and recoveries at 5, 10, 15 and 20 days were reported.

- Stroh (1961). Ova of *Ascaris lumbricoides* were suspended in a container which was placed in an anaerobic digester. The reactor was heated to 27-28°C for an unspecified detention time. Survival of *Ascaris* was monitored for 89 days.

- Ward (1977). The survival of poliovirus type 1 (CHAT) seeded into anaerobically digested sludge and held at 4 and 20°C was examined in this study.

- Ward and Ashley (1976). The effect of anaerobically digested sludge on survival of seeded poliovirus type 1 during incubation at temperatures between 24°C and 28°C was studied in this report.
- Ward and Ashley (1977). The authors examined the effect of an ammonia-N concentration of 8,550 mg/l at pH levels of 7 to 10 on the survival of poliovirus type 1.

b. Full-scale studies:

- Berg and Berman (1980). The relative levels of indicator bacteria (total coliforms, fecal coliforms and fecal streptococci) and viruses were studied during mesophilic (35°C) and what is termed thermophilic digestion (49°C) of sludge at the City of Los Angeles' Hyperion Treatment Plant.
- Cooke *et al.* (1978). Samples from anaerobic digesters at the Dry Creek Plant, Nashville, Tennessee and the Lytle Creek Plant No. 1 and Sinking Creek Plant No. 2, Murfsboro, Tennessee, were analyzed for total coliform bacteria and *Salmonella* sp.
- Jewell *et al.* (1980). A two-year study was conducted at the Binghamton-Johnson City sewage treatment plant, New York, to demonstrate that a simple autoheated aerobic-digestion process could be used to treat sewage sludge. As part of the project, the effectiveness of the experimental thermophilic aerobic system in reducing numbers of indicators, pathogens, viruses and parasites was compared to that of high-rate anaerobic digesters.
- La Conde (1978b). A survey was conducted of eight secondary wastewater treatment plants employing one- or two-stage anaerobic digestion. Levels of fecal coliform bacteria, fecal streptococcus bacteria, *Salmonella* and *Shigella*, and total parasite ova were determined.
- Moore *et al.* (1978). Three sets of sludge samples collected before and after anaerobic digestion at the East Pearl Treatment Plant, Boulder, Colorado, were examined for indigenous enteroviruses. Samples were collected in spring and in middle and late summer. At this site, primary sludge is digested in a heated (37°C) and mixed (gas recirculation) anaerobic digester for a detention time of 40 days. This is followed by digestion in an unheated and unmixed unit for 60 days.
- Stern and Farrell (1977). Reported effects of digestion on total coliform, fecal coliform and fecal streptococcus bacteria (also reported in Berg and Berman, 1980) and *Salmonella* sp. in analyses conducted on sludge from the Hyperion Treatment Plant,

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Los Angeles, California. Two anaerobic digesters were examined, one operated at 35°C and one at 50°C, both with a detention time of 20 days.

c. Studies that report only final density levels:

- Cliver (1975). Examined four anaerobically digested sludge samples collected in December, June, August, and November for enteroviruses and reoviruses. The sludges had been digested anaerobically for 12 days at 30-32°C, then held for eight more days at temperatures upward from 7°-10°C.
- Dudley *et al*. (1980). Two anaerobically digested sludge samples were examined in this study. One sample had undergone digestion in a two-stage high-rate anaerobic system, while the second had undergone standard anaerobic digestion for 20 to 30 days with no mixing or heating. A wide variety of indicator and pathogenic bacteria were enumerated.
- Fox and Fitzgerald (1977). Examined anaerobically digested sludges from four treatment plants within the Metropolitan Sanitary District of Greater Chicago, Illinois. Samples were examined for the presence of ova or cysts of protozoan and metazoan parasites.
- Hess and Breer (1975). A total of 219 sludge samples from 44 different sewage treatment plants were examined for *Salmonella* sp. No conditions of digestion are specified in this report.
- Kenner *et al*. (1971). In studying survival of *Salmonella*, *Ps. aeruginosa*, and fecal coliform bacteria applied to soils, a sample of anaerobically digested sludge was examined prior to land application.
- Lund (1970). Qualitative examination of anaerobically digested sludge (50-60 days at 50°C) for enterovirus was performed in this study.
- Palfi (1972). The virucidal efficiency of anaerobic digestion was assessed at a wastewater-treatment plant in Budapest, Hungary. Picornavirus and reovirus were enumerated in 74 digested sludge samples in 1969, and in 82 samples in 1970. In 1969, digesters were operated at a detention time of 21 days at 30°C. In 1970, sludge was stabilized in a three-stage digester with a total detention time of 40 days at 33°C.

Results

Indicator Bacteria. Levels of indicator bacteria during anaerobic digestion may be examined with respect to detention time and temperature.

The effects of other parameters, such as pH, VS reduction, and sludge type, cannot be evaluated due to the lack of sufficiently controlled data.

a. Detention time -- Survival of *Escherichia coli* was relatd to laboratory-scale digester detention time by McKinney *et al.* (1958). As indicated in Table 5, a greater than 1-log reduction was seen at all detention times, but no correlation of detention time with log reduction is evident. These results are an average, as presented by McKinney *et al.*, of four days of monitoring.

A pilot-scale, four-stage continuous digester with an 11-day detention at 29°C operated by Rawn and Candell (1950) produced a 2.3-log reduction in total coliform bacteria (Table 6). This reduction is similar to that reported by McKinney *et al.* for *E. coli*, an organism that is part of the total coliform group.

Batch digestion for an additional 49 days at 29°C by Rawn and Candell (1950) resulted in an additional reduction of 0.9 log (Table 6) of total coliform bacteria, a much slower rate of decline than found in the initial 11-day period.

Improved reductions of total coliform bacteria were related to longer detention times at three full-scale treatment plants by Cooke *et al.* (1978), as shown in Table 7. At a plant with a 9-day detention, a 1.6-log reduction can be calculated, while at 50-day detention, a 4.0-log reduction is found. Intermediate detention times, ranging from 14 or 15 days to 21 days, found at three other treatment plants yielded reductions of intermediate magnitude. These results are shown in Table 8 (Lue-Hing *et al.*, 1977; Berg and Berman, 1980; and Jewell *et al.*, 1980).

Reduction of *E. coli* on a pilot scale (Table 5) can be seen to conform with the reductions of total coliforms found on full-scale basis (Table 8). Fecal coliform and fecal streptococcus bacteria were also studied by Lue-Hing *et al.* (1977), Berg and Berman (1980), and Jewell *et al.* (1980), as well as by the Sacramento Area Consultants (SAC) in 1979. Reductions of 1 log or more in both groups of organisms are seen between 14 and 21 days detention at 35°C (Tables 9 through 12).

In contrast to the considerable body of data just cited, LaConde (1978) reports results ranging from minimal reductions to significant increase in densities of both fecal coliform and fecal streptococcus bacteria during anaerobic digestion at full-scale plants. Samples were collected from both single-stage anaerobic digesters (Tables 13 and 14) and from two-stage digesters (Tables 15 and 16). In addition to the anomalous reductions reported, the levels of fecal coliform bacteria (per gram dry weight) in raw sludge are a number of orders of magnitude less than levels reported by other authors (Section 2 --Density Levels in Raw Sludge).

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TABLE 5. SURVIVAL OF E. COLI IN HIGH-RATE LABORATORY-SCALE ANAEROBIC DIGESTERS*

Detention Time	Densities (organisms/100 ml)	Log Reduction
Raw sludge	2.0×10^8	-
6 days	5×10^6	1.6
8 days	8×10^6	1.4
10 days	2×10^6	2.0
15 days	1×10^6	2.3
20 days	3×10^6	1.8

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[*] McKinney *et al*. (1958). Temperature 33°-40°C; VS reduction 52-58%. 12-hr feed-and-draw cycle; draw preceded feed for all analyses

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TABLE 6. DENSITY LEVELS OF TOTAL COLIFORM BACTERIA
BEFORE AND AFTER LOW-RATE ANAEROBIC DIGESTION
ON A LABORATORY SCALE AT MESOPHILIC TEMPERATURES

Densities (MPN/100 ml)						Log Reduction	Deten-tion Time (days)	Temperature (°C)	VS Reduction	Authors
Raw			Digested							
Mean[a]	N	Range	Mean	N	Range					
1.5×10^8	NS[b]	NS	6.5×10^5	5	6.2×10^5 $- 7.0 \times 10^5$	2.3	11[c]	29	>38%	Rawn and Candell (1950)
1.5×10^8	NS	NS	2.2×10^5	5	6.0×10^4 $- 6.2 \times 10^5$	2.8	30[d]	29	>38%	Rawn and Candell (1950)
1.5×10^8	NS	NS	9.3×10^4	5	6.0×10^4 $- 2.4 \times 10^5$	3.2	60[d]	29	>38%	Rawn and Candell (1950)

[a] Arithmetic mean of total coliform bacteria density in raw sludge, corrected for an 11% overall seeding with digested sludge.
[b] NS = not specified by author.
[c] Sample taken directly from fourth stage of four-stage digestion tank, 11 days' detention time.
[d] Digestion of the 11-day sample continued under batch conditions for an additional 49 days.

TABLE 7. DENSITY LEVELS OF TOTAL COLIFORM BACTERIA BEFORE AND AFTER
HIGH-RATE ANAEROBIC DIGESTION AT A FULL-SCALE WWTP AT
MESOPHILIC TEMPERATURES

Densities (MPN/100 ml)						Log Reduction	Deten-tion Time (days)	Temperature (°C)	VS Reduction	Authors
Raw[a]			Digested							
Mean[a]	N	Range	Mean	N	Range					
2×10^9 [c]	3	7×10^8- 6×10^9	4×10^7	3	1×10^7- 1×10^8	1.59	9	34	NS[b]	Cooke *et al.* (1978)
2×10^9 [d]	3	1×10^9- 5×10^9	4×10^6	3	3×10^6- 7×10^6	2.63	38	37	NS	Cooke *et al.* (1978)
3×10^9 [e]	3	1×10^9- 4×10^9	2×10^5	3	5×10^4- 7×10^5	4.04	50	37	NS	Cooke *et al.* (1978)

[a] Primary settled sludge.
[b] NS = Not specified.
[c] Metro Nashville's Dry Creek Sewage Treatment Plant.
[d] Sinking Creek No. 2 Murfsboro, Tenn.
[e] Lytle Creek Plant No. 1 Murfsboro, Tenn.

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TABLE 8. DENSITY LEVELS OF TOTAL COLIFORM BACTERIA BEFORE AND AFTER HIGH RATE ANAEROBIC DIGESTION AT FULL SCALE WWTP AT MESOPHILIC TEMPERATURES

Densities (CFU/100 ml)						Log Reduction	Deten-tion Time (days)	Temperature (°C)	VS Reduction	Authors
Raw[a]			Digested							
Mean	N	Range	Mean	N	Range					
1.5×10^8	NS[b]	NS	1.3×10^6	NS	NS	2.06	14-15	35	30%	Lue-Hing *et al*. (1977)
4.3×10^9	10	1.5×10^8 - 1.2×10^{10}	7.1×10^7	10	4.8×10^7 - 1.6×10^8	1.78	20	35	NS	Berg and Berman (1980)
9.2×10^8	5	6.0×10^8 - 1.8×10^9	4.64×10^6	5	1.3×10^5 - 3.7×10^7	2.30	21	35	35%	Jewell *et al*. (1980)
					AVERAGE LOG REDUCTION	2.05				

[a] Mixture of primary and waste activated sludge
[b] NS = Not specified by author

TABLE 9. DENSITY LEVELS OF FECAL COLIFORM BACTERIA BEFORE AND AFTER HIGH RATE ANAEROBIC DIGESTION AT FULL SCALE WWTP AT MESOPHILIC TEMPERATURES

Densities (CFU/100 ml)						Log Reduction	Deten-tion Time (days)	Temperature (°C)	VS Reduction	Authors
Raw[a]			Digested							
Mean	N	Range	Mean	N	Range					
6.4×10^6	NS[b]	NS	1.4×10^5	NS	NS	1.66	14-15	35	30%	Lue-Hing *et al*. (1977)
3.3×10^8	10	5.0×10^6 6.4×10^8	5.4×10^6	10	1.5×10^6- 1.1×10^7	1.79	20	35	NS	Berg and Berman (1980)
3.4×10^7	5	1.5×10^7- 9.6×10^7	3.6×10^5	5	1.1×10^4- 2.0×10^6	1.98	21	35	35%	Jewell *et al*. (1980)
					AVERAGE LOG REDUCTION	1.81				

[a] Mixture of primary and waste activated sludge.
[b] NS = Not specified by author

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TABLE 10. DENSITY LEVELS OF FECAL STREPTOCOCCUS BACTERIA BEFORE AND AFTER HIGH RATE ANAEROBIC DIGESTION AT FULL-SCALE WWTP AT MESOPHILIC TEMPERATURES

Densities (CFU/100 ml)						Log Reduction	Detention Time (days)	Temperature (°C)	VS Reduction	Authors
Raw[a]			Digested							
Mean	N	Range	Mean	N	Range					
4.4×10^6	NS[b]	NS	1.2×10^5	NS	NS	1.56	14-15	35	30%	Lue-Hing *et al.* (1977)
2.8×10^7	10	1.6×10^6- 5.6×10^7	2.2×10^6	10	1.4×10^6- 3.6×10^6	1.10	20	35	NS	Berg and Berman (1980)
3.16×10^7	5	2.2×10^7- 5.0×10^7	3.6×10^5	5	1.1×10^4- 2.2×10^6	1.94	21	35	35%	Jewell *et al.* (1980)
					AVERAGE LOG REDUCTION	1.53				

[a] Mixture of primary and waste activated sludge
[b] NS = Not specified by author

TABLE 11. DENSITY LEVELS OF FECAL COLIFORM BACTERIA BEFORE AND AFTER HIGH RATE ANAEROBIC DIGESTION AT THE SACRAMENTO REGIONAL COUNTY SANITATION DISTRICT AT MESOPHILIC TEMPERATURES

Densities (organisms/GDW)						Log Reduction	Detention Time (days)	Temperature (°C)	VS Reduction	Author
Raw[a]			Digested							
Mean	N	Range	Mean	N	Range					
3.03×10^7	3	NS[b]	1.09×10^6	4	NS	1.44[c]	18-21	35-37	NS	Sacramento Area Consultants (1979)
2.8×10^6	4	2×10^5 - 9.2×10^7	1.3×10^4	4	1.8×10^2- 1.9×10^5	2.33[d]	18-21	35-37	NS	Sacramento Area Consultants (1979)
					AVERAGE LOG REDUCTION	1.89				

[a] Mixture of primary and waste activated sludge
[b] NS = Not specified by author
[c] 1976 data
[d] 1977 data

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TABLE 12. DENSITY LEVELS OF FECAL STREPTOCOCCUS BACTERIA BEFORE AND AFTER HIGH RATE ANAEROBIC DIGESTION AT THE SACRAMENTO REGIONAL COUNTY SANITATION DISTRICT AT MESOPHILIC TEMPERATURES

Densities (organisms/GDW)						Log Reduction	Detention Time (days)	Temperature (°C)	VS Reduction	Author
Raw[a]			Digested							
Mean	N	Range	Mean	N	Range					
3.5×10^7	3	NS [b]	1.23×10^6	4	NS	1.45[c]	18-21	35-37	NS	Sacramento Area Consultants (1979)
1.5×10^6	4	2.7×10^5-1.3×10^7	6.9×10^4	4	6×10^2-7.2×10^5	1.33[d]	18-21	35-37	NS	Sacramento Area Consultants (1979)
					AVERAGE LOG REDUCTION	1.39				

[a] Mixture of primary and waste activated sludge
[b] NS = Not specified by author
[c] 1976 data
[d] 1977 data

TABLE 13. DENSITY LEVELS OF FECAL COLIFORM BACTERIA BEFORE AND AFTER ANAEROBIC DIGESTION AT FULL SCALE WWTP AT MESOPHILIC TEMPERATURES

Densities (MPN/GDW)						Log Reduction	Detention Time (days)	Temperature (°C)	VS Reduction	Sludge type	Author
Raw			Digested								
Mean	N	Range	Mean	N	Range						
1.1×10^1	2	0-1.1×10^2	1.7×10^4	2	1.6×10^2-1.9×10^6	Increase	45	35	NS[a]	Mixture of 1° + trickling filter	LaConde (1978)[b]
1.8×10^2	2	0-3.1×10^4	1.6×10^2	2	0-2.7×10^4	0.05	14	35	NS	Mixture of 1° + trickling filter	LaConde (1978)[c]
5.0×10^4	2	3.2×10^3-7.7×10^5	2.0×10^5	2	3.5×10^3-1.1×10^7	Increase	30	35	NS	Mixture 1° + waste activated sludge	LaConde (1978)[d]

[a] NS = Not specified
[b] Frankfort, IN WWTP
[c] Kendallville, IN WWTP
[d] Columbus, IN WWTP

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TABLE 14. DENSITY LEVELS OF FECAL STREPTOCOCCUS BACTERIA BEFORE AND AFTER ANAEROBIC DIGESTION AT FULL SCALE WWTP AT MESOPHILIC TEMPERATURES

Densities (MPN/GDW)						Log Reduction	Detention Time (days)	Temperature (°C)	VS Reduction	Sludge type	Author
Raw			Digested								
Mean	N	Range	Mean	N	Range						
8.5×10^{6}	2	1.5×10^{5}- 4.8×10^{8}	4.3×10^{7}	2	1.9×10^{6} - 9.6×10^{8}	Increase	45	35	NS[a]	Mixture of 1° + trickling filter	LaConde (1978)[b]
5.4×10^{6}	2	2.4×10^{6}- 1.2×10^{7}	7.3×10^{6}	2	4.4×10^{6}- 1.2×10^{7}	Increase	14	35	NS	Mixture of 1° + trickling filter	LaConde (1978)[c]
1.1×10^{5}	2	1.6×10^{3}- 8.0×10^{7}	1.2×10^{6}	2	1.3×10^{5}- 1.2×10^{7}	Increase	30	35	NS	Mixture of 1° + waste activated sludge	LaConde (1978)[d]

[a] NS = Not specified by author
[b] Frankfort, In. WWTP
[c] Kendallville, In. WWTP
[d] Columbus, In. WWTP

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TABLE 15. DENSITY LEVELS OF FECAL COLIFORM BACTERIA BEFORE AND AFTER TWO STAGE ANAEROBIC DIGESTION AT FULL SCALE WWTPs AT MESOPHILIC TEMPERATURES

Densities (MPN/GDW)						Log Reduction	Detention Time (days)	Temperature (°C)	VS Reduction	Sludge type	Author
Raw			Digested								
Mean	N	Range	Mean	N	Range						
$8.3x10^1$	2	$5.6x10^1$- $1.2x10^2$	$1.2x10^3$	2	$4.7x10^2$- $3.1x10^3$	Increase	30+90	35	NS[a]	Mixed trickling filter + 1°	LaConde (1978)[b]
$5.7x10^2$	2	$3x10^2$- $1.1x10^3$	$8.5x10^2$	2	$7.8x10^2$- $9.3x10^2$	Increase	20	35	NS	Mixed waste activated + 1°	LaConde (1978)[c]
$1.1x10^4$	2	$3.0x10^3$- $3.8x10^4$	$2.5x10^3$	2	$1.6x10^3$- $3.8x10^3$	0.64	21 + 21-60	35	NS	Mixed trickling filter + 1°	LaConde (1978)[d]
$3.0x10^3$	2	$5.6x10^2$- $1.6x10^4$	$7.3x10^5$	2	$2.2x10^5$- $2.4x10^6$	Increase	10 + 20	35	NS	Mixed waste activated + 1°	LaConde (1978)[e]
$3.6x10^6$	2	$1.1x10^5$- $1.2x10^6$	$4.7x10^5$	2	$3.0x10^4$- $7.5x10^6$	0.88	150 + 100	35	NS	Mixed waste activated + 1°	LaConde (1978)[f]

[a] NS = Not specified by author
[b] Macon, Ga. WWTP
[c] Springfield, Mo. WWTP
[d] Hopkinville, Ky. WWTP
[e] Wilmington, Ohio WWTP
[f] Chippewa, Wi. WWTP

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TABLE 16. DENSITY LEVELS OF FECAL STREPTOCOCCUS BACTERIA BEFORE AND AFTER TWO STAGE ANAEROBIC DIGESTION AT FULL SCALE WWTP AT MESOPHILIC TEMPERATURES

Densities (MPN/GDW)						Log Reduction	Detention Time (days)	Temperature (°C)	VS Reduction	Sludge type	Author
Raw			Digested								
Mean	N	Range	Mean	N	Range						
$1.7x10^6$	2	$1.9x10^4$- $1.5x10^8$	$6.1x10^6$	2	$5.3x10^5$- $6.9x10^7$	Increase	30 + 90	35	NS[a]	Mixed trickling filter + 1°	LaConde (1978)[b]
$7.6x10^6$	2	$1.2x10^5$- $4.8x10^8$	$5.9x10^6$	2	$8x10^4$- $4.4x10^8$	0.11	20	35	NS	Mixed waste activated + 1°	LaConde (1978)[c]
$2.5x10^7$	2	$4.0x10^6$- $1.6x10^8$	$1.6x10^6$	2	$3.0x10^4$- $8.3x10^7$	1.19	21+ 21+60	35	NS	Mixed trickling filter + 1°	LaConde (1978)[d]
$2.4x10^7$	2	$1.6x10^6$- $3.5x10^8$	$5.8x10^6$	2	$1.4x10^6$- $2.4x10^7$	0.62	10+ 20	35	NS	Mixed waste activated + 1°	LaConde (1978)[e]
$5.3x10^7$	2	$2.3x10^6$- $1.2x10^9$	$2.3x10^6$	2	$2.3x10^3$- $2.4x10^9$	1.36	150+ 100	35	NS	Mixed waste activated + 1°	LaConde (1978)[f]

[a] NS = Not specified by author
[b] Macon, Ga WWTP
[c] Springfield, Mo. WWTP
[d] Hopkinville, Ky WWTP
[e] Wilmington, Ohio WWTP
[f] Chippewa, Wi WWTP

These results can be explained, most likely, by the method of sample preparation utilized. Essentially, sludge samples were eluted with sterile water and filtered through a series of sieves, with solids discarded at each step. Any bacteria adsorbed to solids would be lost by this method.

b. Temperature -- No data were encountered that compared levels of indicator bacteria during anaerobic digestion at temperatures of 20°C or less to levels at 35°C or more. Comparison of Tables 8, 9, and 10 with Table 17 and Figure 6 indicates that better reductions of indicator bacteria result from anaerobic digestion at 49°C than at 35°C. Digestion at 49°C yielded reductions that were 3 logs, 1 log and 1.5 logs better for total coliform, fecal coliform and fecal streptococcus bacteria, respectively, than those yielded at 35°C (Berg and Berman, 1980).

c. Other indicators -- Table 18 presents density levels of traditional and non-traditional indicator bacteria enumerated by Dudley *et al.* (1980). Levels of the traditional indicators (total coliform, fecal coliform and fecal streptococcus bacteria) are generally within the same orders of magnitude reported by other researchers (Tables 8, 10). The similarity in these results gives credence to the density levels presented for the other, "non-traditional" indicators for which little supporting data is available.

Pathogenic Bacteria. Density levels of *Salmonella* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and tubercle bacteria (*Mycobacterium* sp.) have been studied before and after high-rate anaerobic digestion (Tables 19 through 23).

a. Detention time -- McKinney *et al.* (1958) found that laboratory-scale digesters with a solids'-detention time of 20 days produced a 0.5-log increase in reduction of seeded *Salmonella typhosa* over reductions produced in a 6-day retention reactor. Both reactors were operated on a 24-hour feed-and-draw cycle, with additional *S. typhosa* inoculated every 24 hours (Table 19). In a separate experiment, in which reactors were seeded with only one initial inoculum, rates of reduction can be compared among reactors with 6-, 10-, and 20-day solids-detention times. A small, but still evident, increase in rate occurs in reactors with a 20-day detention time over that in a 6-day detention reactor (Figure 1).

Improved reductions of indigenous *Salmonella* sp. were correlated by Cooke *et al.* (1978) with increased detention times ranging from 9 to 50 days at three full-scale treatment plants (Table 20). The researchers enumerated *Salmonella* sp. by the Alley-Maleney membrane filter technique. Density levels in both raw and digested sludge are generally 3 to 8 logs higher than those reported by other researchers (Table 21). For this reason, while

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TABLE 17. DENSITY LEVELS OF TOTAL COLIFORM, FECAL COLIFORM AND FECAL STREPTOCOCCUS BACTERIA AND SALMONELLA BEFORE AND AFTER THERMOPHILIC ANAEROBIC DIGESTION AT A FULL SCALE WWTP

Densities (CFU/100 ml)						Log Reduction	Detention Time (days)	Temperature (°C)	VS Reduction	Organism
Raw[a]			Digested							
Mean	N	Range	Mean	N	Range					
$4.1x10^9$	11	$1.5x10^8$- $1.2x10^{10}$	$<1.6x10^4$	11	$<6.7x10^2$- $2.9x10^5$	>5.4	20	49	NS[b]	Total coliform[c]
$2.3x10^8$	11	$5x10^6$- $6.4x10^8$	$<1.0x10^4$	11	$<3x10^2$- $9.5x10^4$	>2.7	20	49	NS	Fecal coliform[c]
$2.1x10^7$	11	$1.3x10^6$ $5.6x10^7$	$<1.0x10^4$	11	$<6.7x10^2$- $6.0x10^4$	>3.3	20	49	NS	Fecal streptococcus[c]
$7.5x10^3$	NS	NS	<3	NS	NS	>3.4	20	50	NS	Salmonella[d]

[a] Mixture of primary and waste activated sludge
[b] NS = Not specified by author
[c] Berg and Berman (1980)
[d] Stern and Farrell (1977)

TABLE 18. DENSITY LEVELS OF INDICATOR BACTERIA IN ANAEROBICALLY DIGESTED SLUDGE[a]

Densities (CFU/g TSS)							Digestion Process
				Clostridium Perfringens[b]			
Total Coliform	Fecal Coliform	Escherichia coli	Fecal Streptococcus	Vegetative[c]	Sporulated[d]	Aeromonas hydrophila	
4.5×10^6	4.8×10^5	1.7×10^6	2.4×10^5	2.6×10^7	2.6×10^7	2.9×10^6	Two-stage, high-rate. No temperature or detention time specified.
5.1×10^6	3.1×10^6	2.0×10^5	6.9×10^4	3.1×10^7	4.9×10^6	2.0×10^6	Low-rate digestion for 20-30 days with no mixing or heating.

[a] Dudley *et al.*, (1980)
[b] Densities as MPN/g TSS
[c] At room temperature
[d] At 80°C.

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TABLE 19. DENSITY LEVELS OF SALMONELLA TYPHOSA SEEDED INTO A HIGH-RATE LABORATORY SCALE DIGESTER AT MESOPHILIC TEMPERATURES[a]

Densities (count/100 ml)						Log Reduction	Detention Time (days)	Temperature (°C)	VS Reduction
Initial[b]			Digested[c]						
Mean	N	Range	Mean	N	Range				
3.3×10^7	24	6.3×10^5- 1.3×10^8	2.8×10^6	24	$0-3.1 \times 10^7$	1.07	6	33-40	52.2%
2.9×10^7	24	1.5×10^6- 1.4×10^8	7.8×10^5	24	$0-2.3 \times 10^5$	1.57	20	33-40	58.2%

[a] McKinney et al. (1958). Fed seeded sludge at 24 hr. intervals, withdrew a sample for Salmonella enumeration prior to feed every 24 hr. for 24 days

[b] Inoculated sludge (daily)

[c] After 24 hr. (prior to feed)

TABLE 20. DENSITY LEVELS OF SALMONELLA SPP. BEFORE AND AFTER HIGH RATE ANAEROBIC DIGESTION AT FULL SCALE WWTPs AT MESOPHILIC TEMPERATURES

Densities (MPN/100 ml)[f]						Log Reduction	Detention Time (days)	Temperature (°C)	VS Reduction	Author
Raw[a]			Digested							
Mean	N	Range	Mean	N	Range					
4.0×10^8	3	2×10^8- 9×10^8	7×10^6	3	3.0×10^6- 1.0×10^7	1.75	9	34	NS[b]	Cooke et al. (1978)[c]
3×10^8	3	1×10^8 6×10^8	2×10^5	3	9×10^4- 4×10^5	3.32	38	37	NS	Cooke et al. (1978)[d]
5×10^8	3	4.0×10^8- 5×10^8	5×10^4	3	2×10^4- 1×10^5	3.97	50	37	NS	Cooke et al. (1978)[e]

[a] Primary settled sludge

[b] NS = Not specified by author

[c] Metro Nashville's Dry Creek Sewage Treatment Plant

[d] Sinking Creek No. 2 - Murfsboro, Tenn.

[e] Lytle Creek Treatment Plant No. 1. Murfsboro, Tenn.

[f] Enumerated by the Alley-Malaney membrane filter technique.

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TABLE 21. DENSITY LEVELS OF SALMONELLA SPP., PSEUDOMONAS AERUGINOSA, AND STAPHYLOCOCCUS AUREUS BEFORE AND AFTER HIGH RATE ANAEROBIC DIGESTION AT FULL SCALE WWTP AT MESOPHILIC TEMPERATURES

Densities (MPN/100 ml)						Log Reduction	Detention Time (days)	Temperature (°C)	VS Reduction	Author
Raw[a]			Digested							
Mean	N	Range	Mean	N	Range					
						Salmonella spp.				
$1.2x10^2$	NS[b]	NS	$1.6x10^0$	NS	NS	1.89	14-15	35	30%	Lue-Hing *et al.* (1977)
$7.5x10^3$	5	NS	$6.2x10^1$	5	NS	0.91	21	35	35%	Stern and Farrell (1977)
$3.9x10^2$	NS	$4.1x10^1$- $2.2x10^3$	$4.8x10^1$	NS	$3x10^0$- $1.1x10^3$	2.08	20	36	NS	Jewell *et al.* (1980)
					AVERAGE LOG REDUCTION	1.63				
						Pseudomonas aeruginosa				
$1.0x10^6$	NS	NS	$7.1x10^5$	NS	NS	0.149	14-15	35	30%	Lue-Hing *et al.* (1977)
$1.1x10^2$	5	$1.1x10^0$- $1.2x10^3$	$1.1x10^1$	NS	<3-$7.2x10^1$	1.0	21	35	35%	Jewell *et al.* (1980)
					AVERAGE LOG REDUCTION	.58				
						Staphylococcus aureus				
$1.9x10^4$	NS	NS	$2.5x10^4$	NS	NS	Increase	14-15	35	30%	Lue-Hing *et al.* (1977)

[a] Mixture of primary and waste activated sludge

[b] NS = Not specified by author

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TABLE 22. DENSITY LEVELS OF SALMONELLA AND SHIGELLA SPP. (ENUMERATED SIMULTANEOUSLY) BEFORE AND AFTER HIGH RATE ANAEROBIC DIGESTION AT THE SACRAMENTO REGIONAL COUNTY SANITATION DISTRICT AT MESOPHILIC TEMPERATURES

Densities (MPN/GDW)						Log Reduction	Detention Time (days)	Temperature (°C)	VS Reduction	Author
Raw[a]			Digested							
Mean	N	Range	Mean	N	Range					
ND[b]	3	-	ND	4	-	- [d]	18-21	35-37	NS[c]	Sacramento Area Consultants (1979)
$<4.8\text{x}10^{-1}$	4	ND- $<8.3\text{x}10^{-1}$	$>3.8\text{x}10^{-1}$	4	ND - >5	- [e]	18-21	35-37	NS	Sacramento Area Consultants (1979)

[a] Mixture of primary and waste activated sludge
[b] ND = None detected
[c] NS = Not specified by author
[d] 1976 Data - data not amenable to log reduction
[e] 1977 Data - data not amenable to log reduction

TABLE 23. DENSITY LEVELS OF SEEDED TUBERCLE BACTERIA (MYCOBACTERIUM SP.) DURING LABORATORY SCALE ANAEROBIC DIGESTION*

Digestion period (days)	Tubercle bacteria		Log reduction	Percent VS reduction
	per ml	per gm dry wt.		
1	9×10^4	1.9×10^6	-	0.9
8	3.6×10^4	7.6×10^5	0.40	15.
15	3.5×10^4	7.4×10^5	0.47	27.
18	1.9×10^4	4.1×10^5	0.67	34.
25	2.2×10^4	4.7×10^5	0.61	47.
35	1.4×10^4	3.0×10^5	0.80	59.

*Heukelekian and Albanese (1956). No temperature of digestion specified by author.

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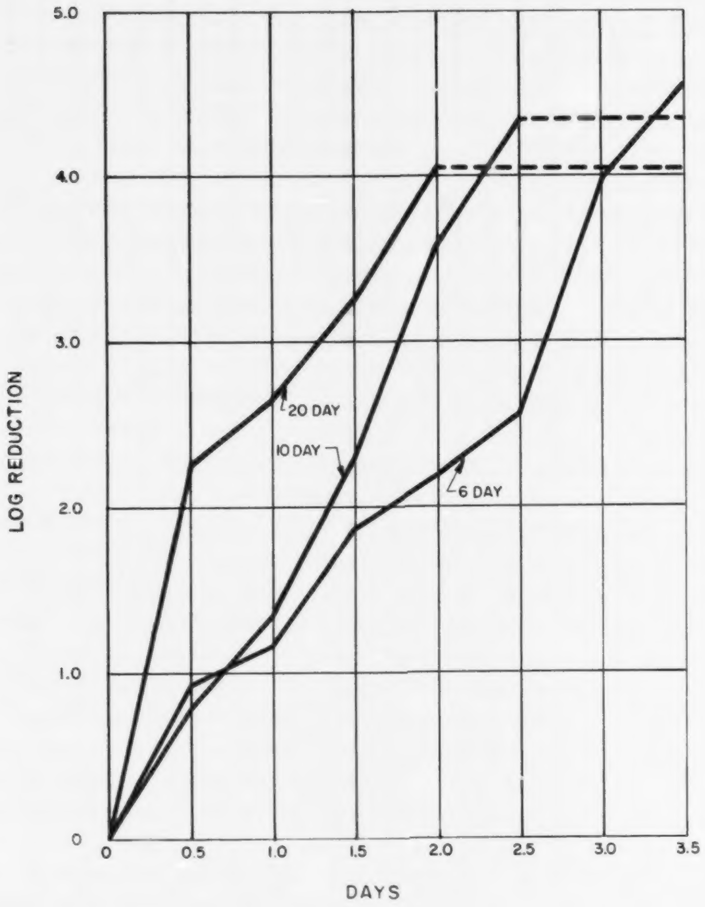

FIGURE I. REDUCTION OF SALMONELLA TYPHOSA INOCULATED INTO ANAEROBIC DIGESTERS AT 6, 10 AND 20 DAY SOLIDS RETENTION.*

* McKINNEY et al., (1958)

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log reductions will be considered valid because the same technique was used throughout the study, density levels will not be included in further discussion. Reductions reported by Lue-Hing *et al.* (1977) and Stern and Farrell (1977), presented in Table 21, fall within the ranges found by Cooke *et al.* The reduction calculated for the data presented by Jewell *et al.* (1980), 0.91-log reduction (Table 21), does not agree with the trends indicated by Cooke *et al.* (1978).

The difficulties inherent in enumerating *Salmonella* and *Shigella* sp. (and in fact, all pathogens present at low density) in sewage sludge is exemplified in data reported by Sacramento Area Consultants or SAC (1979), which are presented in Table 22. These pathogens are present at such low densities compared to indicator bacteria that "catching" them is difficult. SAC (1979) failed to detect these pathogens in either raw or digested sludge in all but two samples collected in 1976 and 1977. In order to report a more meaningful number than "not detected", SAC calculated the geometric mean of the detection limit for each group of samples collected (per site) in 1977 and reported one-half of that number.

In contrast to the "below detectable limits" results previously discussed, Hess and Breer (1975) isolated *Salmonella* bacteria from 91.8 percent of a total of 56 raw sludge samples and 81.9 percent of a total of 136 anaerobically digested samples. Densities were approximately 4 x 10^3 per 100 ml in raw sludge and 1 x 10^2 per 100 ml in digested sludge.

Reductions of less than 1 log in densities of *Mycobacterium tuberculosis* were found by Heukelekian and Albanese (1956) after 35 days of batch digestion (Table 23).

Density levels of *Mycobacterium* sp. reported by Dudley *et al.* (1980) enforce the notion that Mycobacteria are more resistant to the digester environment than other vegetative cells. While density levels in raw sludge were not examined, 10^7 CFU/g total suspended solids of *Mycobacterium* sp. were enumerated by Dudley and co-workers. The *Mycobacterium* count represents 34 percent of the total aerobic bacteria count reported. Results of the team's analyses for *Mycobacterium* sp. and a number of other pathogens are presented in Table 24.

Viruses. Survivals of enteric viruses (the enteroviruses or picornaviruses and reoviruses) as well as bacteriophages (MS-2) have been examined with relation to detention time -- Sanders *et al.* (1979); temperature -- Sanders *et al.* (1979), Eisenhardt *et al.* (1977), Fenters *et al.* (1979), Ward and Ashley (1976), Ward (1977), and Berg and Berman (1980); type of virus -- Bertucci *et al* (1977); effect of ammonia-nitrogen -- Fenters *et al.* (1979) and Ward and Ashley (1977).

All of the studies listed above, with the exception of Berg and Berman (1980), were conducted on a laboratory-scale basis using seeded enteric virus. Additionally, in all of the studies involving draw-and-fill opera-

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TABLE 24. DENSITY LEVELS OF PATHOGENIC BACTERIA IN ANAEROBICALLY DIGESTED SLUDGE COLLECTED FROM FULL-SCALE WWTPs [a]

Densities (CFU/g TSS)									
Total Aerobic Count	*Mycobacterium* sp.	*Staphlyococcus* sp.	*Salmonella* sp.	*Shigella* sp.	*Klebsiella pneumoniae*	*Proteus morganii*	*Yersinia enterocolitica*	*Pseudomonas aeruginosa*	Digestion Process
4.8×10^8	4.0×10^7	$<7.9 \times 10^4$ (ND)[b]	$\geq 2.4 - <24$	ND	1.6×10^5	ND	ND	7.9×10^4	Two stage high rate. No temperature or detention time specified
1.2×10^8	4.1×10^7	$<6.7 \times 10^4$ (ND)	≥ 2.0	≥ 20	6.1×10^5	2.0×10^5	2.0×10^5	ND	Low rate digestion for 20-30 days with no mixing or heating

[a] Dudley et al. (1980)
[b] ND = None/Not detected.

tion of an anaerobic digester (Sanders et al., 1979; Eisenhardt et al., 1977; and Bertucci et al., 1977), virus-inoculated sludge was diluted by successive additions of non-inoculated sludge. To aid the reader in comparing these three studies in the review which follows, Table 25 presents operating parameters and experimental methodology utilized.

a. Detention time -- Design detention time plays a secondary role to temperature in effecting the reduction of the only enteric virus studied in this report, poliovirus type 1 (Sanders et al., 1979). As shown in Figure 2, similar rates (log reduction per day) of decline can be seen in reactors operating at detention times of 5, 10 or 15 days, at 37°C, 34°C and 50°C. No apparent trends link density of organisms recovered with the reactor (solids) detention time. It is, however, quite apparent that the longer that viruses are kept in contact with the anaerobic environment in the temperature range studied, the fewer viruses are recovered.

Actual digestion in full-scale plants, where the process is subject to problems such as overloading and short-circuiting, cannot guarantee the reductions in enterovirus predicted by laboratory- and pilot-scale studies. In comparison to the 1- to 2- log reduction within 5 days reported by Sanders et al. (1979), Moore et al. (1977) found reductions of less than 1 log in total enterovirus in full-scale continuous digesters with a 40-day detention time (Table 26). In this study, digestion was followed by thickening for an additional 60 days, yielding somewhat higher reductions overall in viral titer. Lund (1970), also reported poliovirus (not quantitative) in sludge samples that had been digested for 50 to 60 days at 50°C.

b. Temperature -- In two laboratory-scale studies (Sanders et al. 1979, Eisenhardt et al., 1977) that have compared virus survival to temperature in an actual digester environment, temperatures from a range of only 32 to 50°C were examined. Appreciable increases in the rate of inactivation occur with even a 3°C increase in temperature from 32 to 35°C (Table 27) and from 34 to 37°C (Figure 2).

Sanders et al. (1979) observed that loss of poliovirus at 34° and 37°C took place at two different rates: an initial rapid rate of 1-2 logs per day in the first 24 hours and a slower, steady rate (secondary rate, k_2) of 0.2 to 0.4 logs per day thereafter. The secondary rate was calculated using regression analysis. A comparison of these secondary rates at 34, 37 and 50°C is presented in Figure 2. It can be seen that an increase in temperature to 50°C produces a >2-log reduction per day, compared to 0.2-0.4 log/day at 34° to 37°C.

Studies have followed reduction of virus seeded into and incubated in anaerobically digested sludge (Ward and Ashley, 1976; Ward, 1977) or sludge supernate (Fenters et al., 1979); in these stu-

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TABLE 25. COMPARISON OF METHODS AND EXPERIMENTAL CONDITIONS FOR RECENT STUDIES ON VIRUS RECOVERY DURING LABORATORY-SCALE ANAEROBIC DIGESTION

Type Digester	Continuous (fill & draw)	Continuous (fill & draw)	Continuous (fill & draw)
Digester Detenion Time	5,10,15 da	14 da	35 da
Virus Detention	5,10,15 da	2 da	4-14 da
Mixing	Continuous	Continuous	Continuous
Volatile Solids Reduction	35 - 44%	30%	NS[a]
Temperature	34,37,50°C	35°C	32 & 35°C
Virus	Polio 1 (Chat)	Polio 1; Coxsackie A9, B4; Echo 11; MS-2 coliphage	Coxsackie B3
Virus Addition	Incorporated into MLSS	Mixed with sludge (aer. for 15 min.)	Added to digester mixed for 5 min.
Virus levels Corrected for dilution with unseeded sludge	Yes	NS	NS
Preparation for Assay	Direct Plate	Dilution in 5%FCS, 3%BE; 1% gelatin, sonication for 20-min direct assay	Direct plate after ether treatment
Units reported in	Fraction recovered or rate of loss (%/day)	% virus inactivation	Log $TCID_{50}$/0.1 ml
pH	6.0 - 7.2	7.2 - 7.4	7.0
Author	Sanders et al. 1979	Bertucci et al. 1977	Eisenhardt et al. 1977

a NS= Not specified by author

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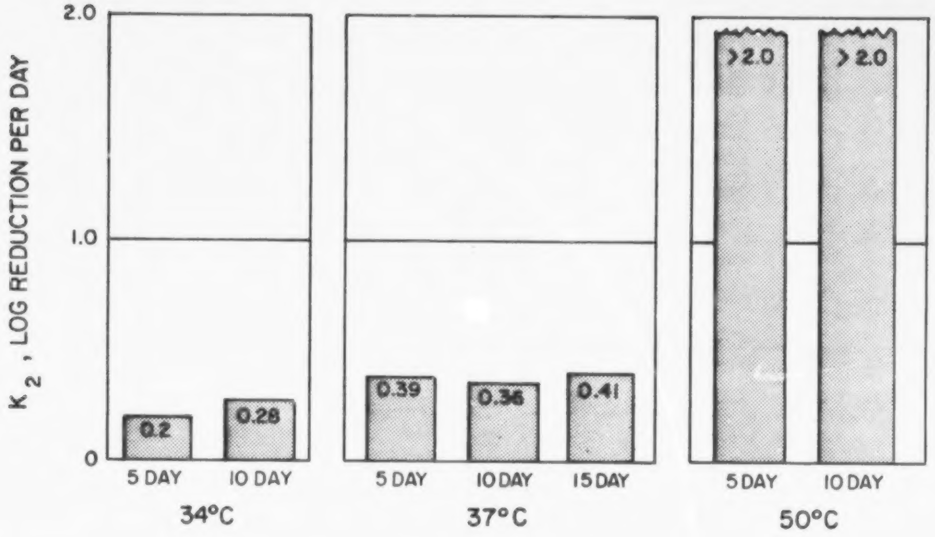

FIGURE 2. SECONDARY RATE OF LOSS OF POLIOVIRUS, TYPE I, CHAT DURING LABORATORY SCALE ANAEROBIC DIGESTION.*

* SANDERS et al., 1979 (RATE OF REDUCTION IS CALCULATED BASED ON REGRESSION ANALYSIS FOLLOWING THE INITIAL 24 HOURS POST INOCULATION)

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TABLE 26. LOG REDUCTION OF ENTEROVIRUS DURING TWO-STAGE ANAEROBIC DIGESTION AT A FULL SCALE WWTP*

Sample	Digester 40 da detention 37°C, gas mix	Thickener 60da detention unheated, unmixed	Total Log Reduction
1	1.10	.03	1.13
2	0.54	.14	.68
3	.89	.06	.95
Mean Log Reduction	0.84	.08	0.92

*Adapted from Moore et al. (1978) Primary raw feed sludge. Density levels not specified by author

TABLE 27. LOG REDUCTION OF SEEDED COXSACKIE, TYPE B3 DURING ANAEROBIC DIGESTION*

Time (days)	32°C	35°C
1	-	1.78
2	-	4.00
3	-	5.40
4	-	5.78
7	3.55	-
14	5.39	-

*Eisenhardt et al. (1977). Detention time 35 days; pH 7.0; VS reduction not specified by author; density levels not specified by author

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dies, researchers have again found increased rates of decline with increased temperatures. Results reported by Ward and Ashley (1976) are presented in Table 28. These results indicate a much higher rate of inactivation than would be expected based on Sanders et al. The experimental design used in these experiments does not address many important parameters that, as part of the digestion process, have an unknown--but potentially significant--effect on virus survival.

Temperature effects noted on a laboratory scale are also found in full-scale plants where continuous inoculations of fresh sludge and virus prevent the production of effluent with the low levels of viruses reported in laboratory-scale. An approximate 1-log reduction in indigenous enterovirus occurs in 20 to 21 days at 35°C (Berg and Berman, 1980; Jewell et al., 1980), while at 49°C, a greater than 2.67-log reduction occurs in 20 days (Table 29).

c. Type of virus -- Statistically significant differences in the survival of four different types of seeded enteroviruses and one bacteriophage during laboratory-scale anaerobic digestion were reported by Bertucci et al. (1977), as shown in Figure 3. Echovirus type 11 was found to be the most resistant of the three populations that resulted from an analysis of covariance (0.6-log reduction per day). Coliphage MS-2, poliovirus type 1 and coxsackie type B4 all had intermediate survivals, and coxsackie type A9 was least resistant. All viruses in the latter two groups were reduced by greater than 1 log in 24 hours of digestion.

d. Ammonia-nitrogen -- Ammonia-N has been correlated with reduction of enterovirus by Ward and Ashley (1977) and Fenters et al. (1979). In both studies, virus was suspended in varied concentration of ammonia and buffer solution at concentrations from zero ammonia nitrogen to 10 times that found in anaerobically digested sludge (8,550 mg/l). Ward and Ashley (1977) reported that an appreciable virucidal effect could occur only at pH values of 8 or greater. At a pH of 9.5, greater than 6-log reductions of enterovirus occur within 24 hours. At pH 7, both Fenters et al. (1979) and Ward and Ashley (1977) found that while reductions were still directly related to ammonia concentrations, reductions were not as dramatic (Table 30).

A number of studies conducted at full-scale treatment plants do not fit into the categories just mentioned. Palfi (1972) examined density of enteric viruses surviving anaerobic digestion under two different operating regimes. In 1959, digesters were operated in a single-stage mode at 30°C, using a 21-day detention time. Palfi was able to isolate one or more virus strains in 28 out of 74 digested sludge samples, or 38 percent of the samples. Among virus types, reoviruses and echoviruses were found to be present in highest numbers. In his 1970 study, Palfi studied sludge exposed to three stages of digestion over a total detention time

TABLE 28. REDUCTION OF SEEDED POLIOVIRUS TYPE 1 CHAT DURING INCUBATION OF ANAEROBICALLY DIGESTED SLUDGE*

Time (days)	Temperature		
	4°C	20°C	28°C
1	-	-	1.24
3	-	2.19	-
5	1.42	4.57	>5.52
log reduction per day	0.28	0.91	>1.10

*Ward and Ashley (1976) and Ward (1977)

TABLE 29. DENSITY LEVELS OF ENTEROVIRUSES BEFORE AND AFTER HIGH RATE ANAEROBIC DIGESTION AT FULL SCALE WWTP AT 35 AND 49°C

Densities (PFU/100 ml)						Log Reduction	Deten-tion Time (days)	Temperature (°C)	VS Reduction	Author
Raw[a]			Digested							
Mean	N	Range	Mean	N	Range					
433	9	152-1060	19	9	4-100	1.36	21	35	35%	Jewell *et al.* (1980)
1545	11	380-11,600	138	10	30-410	1.05	20	35	NS[b]	Berg and Berman (1980)
AVERAGE LOG REDUCTION						1.21				
1545	11	380-11,600	<3.3	11	<1.7-16.7	>2.67	20	49	NS	Berg and Berman (1980)

[a] Mixture of primary and waste activated sludge
[b] NS = Not specified by author

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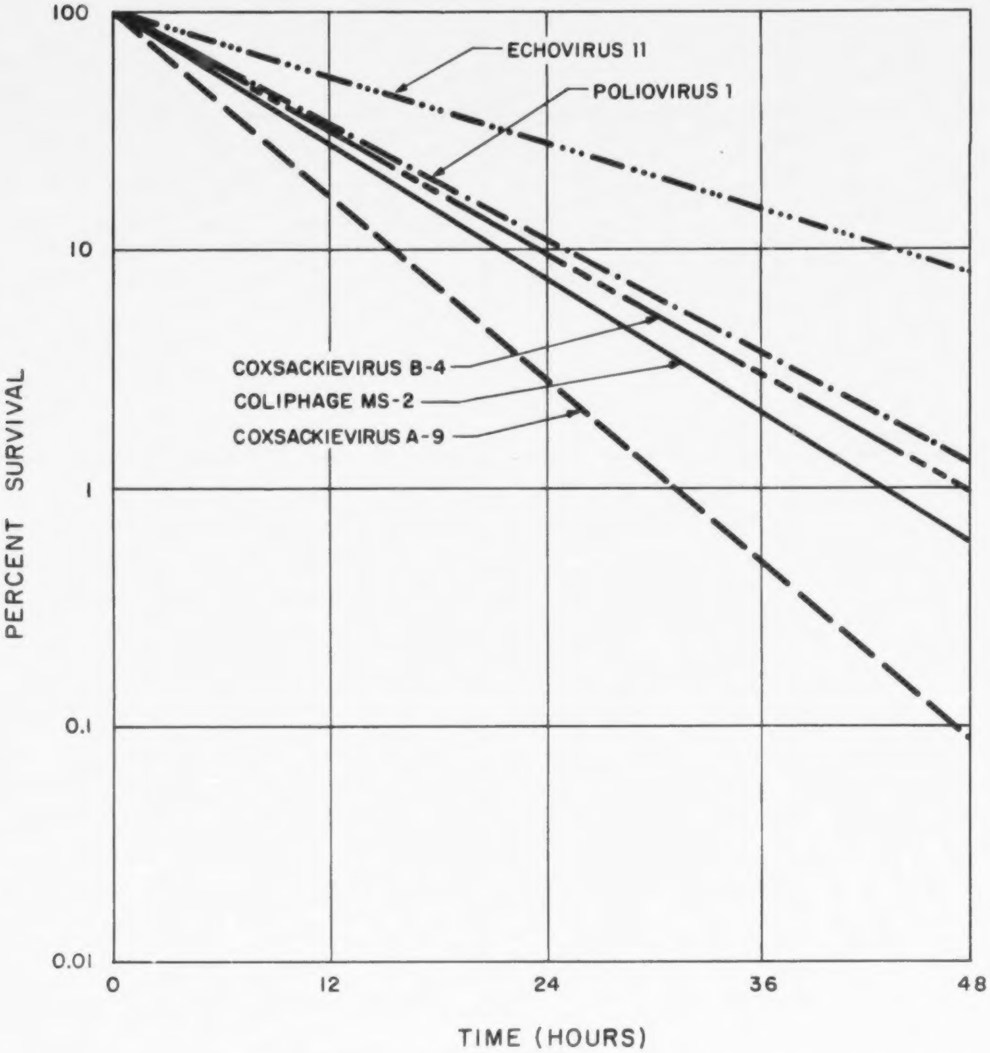

FIGURE 3. REDUCTION OF SEEDED VIRUSES DURING LABORATORY SCALE ANAEROBIC DIGESTION. *

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* BERTUCCI et al. (1977), 35° C, 14 DAYS DETENTION, ~30% VS REDUCTION (REGRESSION DERIVED CURVES)

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TABLE 30. REDUCTION (PER DAY) OF POLIOVIRUS TYPE 1 IN AMMONIA

Organism	Ammonia-N*	pH	Log Reduction Per Day	Author
Poliovirus Type 1 (Sabin)	0 mg/l	7.0	0.25	Fenter et al. (1979)**
	500 mg/l	7.0	0.33	
	750 mg/l	7.0	0.34	
	1000 mg/l	7.0	0.30	
	1500 mg/l	7.0	0.42	
Poliovirus Type 1 (Chat)	8550 mg/l	7.0	0.17	Ward and Ashley (1977)**
(Mahoney)	8550 mg/l	7.0	0.28	
Type 1 (Chat)	8550 mg/l	9.5	>6	Ward and Ashley (1977)***
(Mahoney)	8550 mg/l	9.5	>6	

*NH^4Cl

**Slope of regression curve. 7 day incubation @ 35°C

***Incubated at 21°C for 24h.

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of 40 days. Sludge was first held in two primary digesters for 20 and 10 days, respectively, and then fed to a secondary digester for 10 days' detention at 33°C without mixing. Under these conditions, 13 of 82 sludge samples, or 15 percent, proved positive for virus. A geometric mean for the 82 samples of 1.2 MPNCU per 100 ml (Most Probable Number of Cytopathogenic Units per 100 ml) with a range of 0 to 19 MPNCU/100 ml can be calculated for these data.

In a study by Cliver (1975), a blend of thickened primary and secondary sludge was digested anaerobically for 12 days at 30°C and then held for 8 days at 7-10°C. While initial virus densities were not enumerated, enterovirus and reovirus levels in samples collected following this treatment are presented in Table 31.

Parasites. Because of the differences in response of parasitic organisms to anaerobic digestion -- which are relatively unknown at the species level --and the variations in operating conditions used to examine parasite survival during the digestion process, this section will be approached on a taxonomic basis, examining effects of digestion on each particular species, rather than examining effects of specific operating variables on parasitic organisms as a total group. To aid the reader in comparing the literature discussed, Table 32 presents a phylogenetic listing of organisms, along with the author who examined or encountered them, and pertinent operating parameters.

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a. Protozoan parasites -- Seeded cysts of *Entamoeba histolytica*, the agent of amoebic dysentery in humans, were found by Cram (1943) to be less resistant to the effects of low-rate anaerobic digestion on a laboratory scale than were ova of *Ascaris lumbricoides* or *Ancylostoma canium* (discussed below). While no initial levels of the ova were given by the author, she reports that no viable cysts were found in batch conditions after 12 days at 20°C, 10 days at 30°C, and 4 days at temperatures ranging from -12°C to 19°C.

Only non-viable cysts of *E. histolytica* were found in raw (primary and waste activated) sludge collected from the Binghamton-Johnson City WWTP, New York; no cysts were found after digestion (Jewell *et al.,* 1980).

b. Metazoan parasites

(1) Tapeworms -- Silverman and Guiver (1960) seeded ova of *Taenia saginata* into primary sludge that was incubated under anaerobic conditions on a laboratory scale at 35°C. No viable ova were found after 5, 10, 15 or 20 days incubation when examined microscopically and utilizing *in vitro* testing.

(2) Flatworms -- Ova of seeded *Schistosoma japonicum* were found to be destroyed within 26 days at 24-29°C (Jones *et al.,* 1947), while at 7-18°C, viable miracidia (first larvae) were

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TABLE 31. LEVELS OF ENTEROVIRUS AND REOVIRUS IN ANAEROBICALLY DIGESTED SLUDGE FROM A FULL-SCALE TREATMENT PLANT*

	Enteroviruses	Reoviruses
Mean	5.9	8
Range	1.3 - 17.	6. - 17.
N	4	3

*Cliver, 1975. Densities in PFU/100 ml.

TABLE 32. A SUMMARY, BY TAXA, OF PARASITIC ORGANISMS STUDIED DURING ANAEROBIC DIGESTION

TAXA	OPERATING PARAMETERS					AUTHOR
	Temperature	Mode	Digester Detention	Organism Detention	Scale	
a) Protozoa						
Entamoeba histolytica	20°, 30°C	Batch	-	160da	Lab	Cram, 1943[a]
	35°C	Continuous	21 da	21da	Full	Jewell, 1980
b) Metazoa						
Platyhelminthes						
Cestoda (tapeworms) *Taenia saginata* (beef tapeworm)	35°C	Batch	-	20da	Lab	Silvermann & Guiver, 196
Trematoda (flatworms) *Schistosoma japonicum* (blood fluke)	24 - 29°C	Fill & draw	NS[b]	26da	Lab	Jones *et al.,* 1947[a]
	7 - 18°C	Fill & draw	NS	70da	Lab	Newton *et al.,* 1948[a]
Nemathelminthes						
Nematoda *Ascaris lumbricoides* (round worm)	20, 30°C	Batch	-	160da	Lab	Cram,,1943[a]
	38°C	Batch	-	21da	Lab	Fitzgerald & Ashley, 197
	35°C	Continuous	21 da	21da	Full	Jewell *et al.,*1980
	28°C	NS	NS	89da	Full	Stroh, 1979[a]
	35°C	NS	variable	-	Full	LaConde, 1978
	35, 45°C	Continuous	15 da	15 da	Lab	Reimers *et al*., 1980
Toxacara sp. (dog and cat ascarid)	35°C	Continuous	-	21da	Full	Jewell *et al.,* 1980
	35°C	NS	variable	-	Full	LaConde, 1978
	35, 45°C	Continuous	15 da	15 da	Lab	Reimers et al., 1980
Trichuris trichura (whipworm)	35°C	Continuous	-	21da	Full	Jewell *et al.,*1980
Ancylostoma canium (dog hookworm)	20, 30°C	Batch	-	160da	Lab	Cram, 1943[a]
Trichinella spiralis	35°C	Fill & draw	14 da	96hr, 16da	Lab	Fitzgerald & Prakasam, 1978[a]

[a] Inoculation experiments

[b] NS = Not specified by author

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found until 63 days of laboratory-scale digestion. Results of these experiments are presented in Table 33.

(3) Nematodes -- Anaerobic digestion at 35°C for 15-20 days has been reported by Cram (1943), Fitzgerald and Ashley (1977), Reimers *et al.* (1980), and Stroh (1979) to have only minor effects on the reduction of viable ova of seeded *Ascaris* sp. At 45°C, however, log reductions of greater than 2 in levels of seeded *A. lumbricoides* var *suum* and *Toxocara canis* occur within two days (Reimers *et al.*, 1980).

Average percent viability vs. length of digestion in batch units reported by Cram is presented in Figure 4.

Stroh (1979), suspended ova of *Ascaris lumbricoides* in full scale digesters, operated at 28-29°C. Reductions of viable ova were only 0.29 logs after 80 days. Reductions of 0.19 to 0.28 logs were found in 3 of 4 sludge samples subjected to conditions simulating anaerobic digestion (at 38°C for 21 days) by Fitzgerald and Ashley (1977). The fourth sample, digested at the same temperature and for the same time, achieved a greater than 2 log reduction in inoculated *Ascaris*. No explanation for this lethal effect was offered by the authors. Similar reductions of less than 1 log in numbers of ova of *A. lumbricoides* var *suum* and *T. canis* were recorded by Reimers *et al.* (1980) in laboratory scale digesters operated at 35°C. Viable ova of *A. lumbricoides* var *suum* and *T. canis* were reduced by 0.23 and 0.25 logs, respectively, after 15 days digestion. The same researchers found greater than 2 log reductions of these organisms at 45° and 55°C within 2 days. Extended duration of digestion--to 30 days--was found, at least in batch digesters on a laboratory scale, to effectively reduce levels of seeded *A. lumbricoides*. Kruse (1977) reports a 3 log (99.9%) reduction of *A. lumbricoides* after 30 days digestion at 38°C.

A number of nematode species were isolated from raw and anaerobically digested sludge collected from the Binghamton-Johnson City sewage-treatment plant by Jewell *et al.* (1980). As indicated in Table 35, digestion at 35°C for approximately 21 days did not affect the survival of total parasite ova, including *Ascaris* sp. ova. Similarly, Reimers *et al.* (1980) found that anaerobic digestion at full-scale plants, with no temperature or retention time specified, was ineffective in causing the reduction of parasites and actually resulted in an increase in the number of parasites, due to concentration. Parasites encountered by Jewell *et al.* (1980) in order of frequency of isolation were: *Toxocara* sp., *Trichuris* sp. and *Ascaris* sp. *Ascaris* ova and *Toxocara* sp. were also the most commonly isolated parasites in raw and digested sludges

TABLE 33. SURVIVAL OF SEEDED *SCHISTOSOMA JAPONICUM* OVA DURING LABORATORY-SCALE ANAEROBIC DIGESTION*

	7°-18°C				24°-29°C			
Time (days)	Mean**	N	Range	Log Reduction	Mean	N	Range	Log Reduction
0	165	2	155-175	-	383	4	260-620	-
7	120	2	120-121	0.14	147	4	14-462	0.42
14	80	2	56-114	0.31	65	4	8-285	0.77
21	70	2	51-96	0.37	12	4	0-65	1.5
26	-	-	-	-	0	2	0	>2.6
28	34	2	26-45	0.69	-	-	-	-
56	2	2	0-6	1.9	-	-	-	-
63	2	2	0-5	1.9	-	-	-	-
70	0	2	0	>2.2	-	-	-	-
	Newton *et al*. (1948)				Jones *et al*. (1947)			

* Fresh sludge (mixture of primary and waste activated sludge) added daily, mixed once daily and prior to sample collection. VS reduction, reactor detention time not specified by author.

** All densities in miracidia/100 ml

TABLE 34. REDUCTION IN *ASCARIS LUMBRICOIDES* SUSPENDED IN A FULL-SCALE ANAEROBIC DIGESTER AT 28-29°C*

Time (days)	Log reduction
34	0.04
47	0.06
61	0.17
71	0.19
89	0.29

* Stroh (1979). Density levels, reactor detention time, VS reduction not specified by author.

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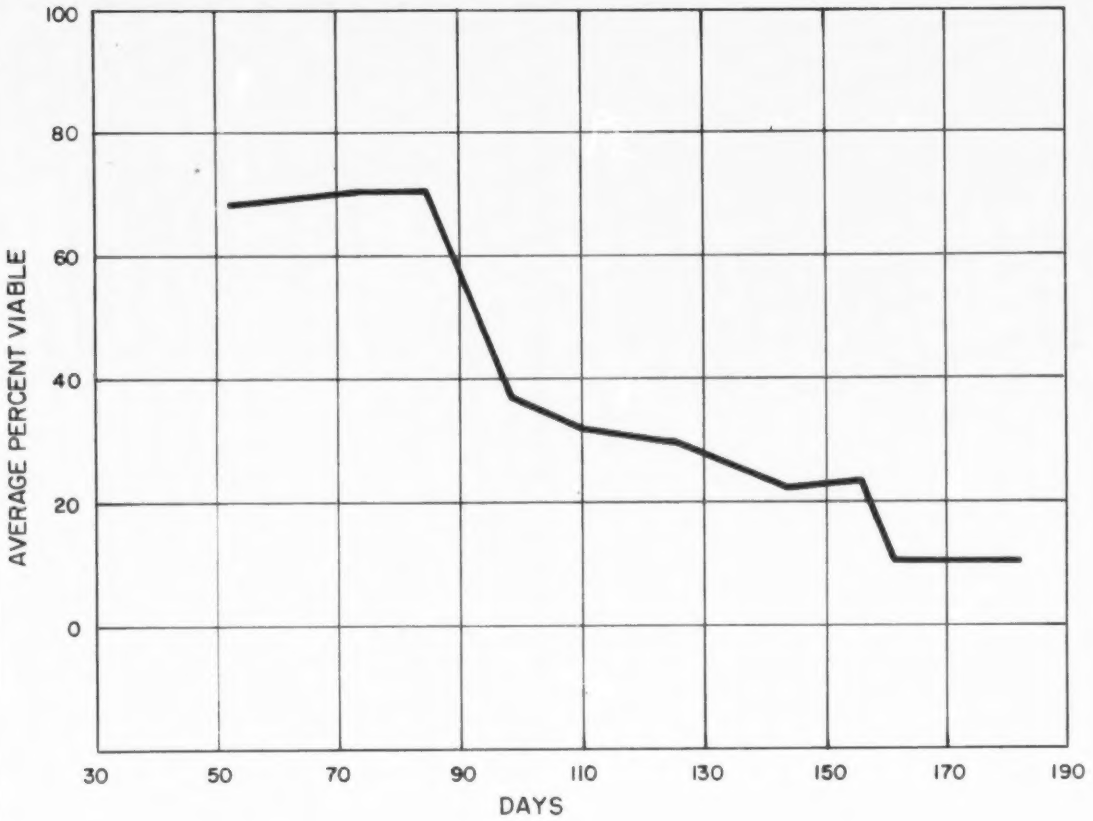

FIGURE 4. VIABILITY OF SEEDED ASCARIS LUMBRICOIDES OVA DURING LABORATORY SCALE DIGESTION AT APPROXIMATELY 20°-30°C OR GREENHOUSE TEMPERATURES.*

*CRAM (1943) AVERAGE OF 18 DIGESTION RUNS

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TABLE 35. DENSITY LEVELS AND PERCENT VIABILITY OF TOTAL PARASITE OVA[a] BEFORE AND AFTER ANAEROBIC DIGESTION AT FULL SCALE WWTP AT MESOPHILIC TEMPERATURES

Densities (total parasite ova/100 ml)						Log Reduction	Detention Time (days)	Temperature (°C)	VS Reduction	Author
Raw[b]			Digested							
Mean	N	Range	Mean	N	Range					
9 68% viable[c]	6	6 - 17 11-100% viability	9 75% viable[c]	6	6-20 38-100% viability	0	21	35	35%	Jewell *et al*. (1980)

[a] Organism recovered: *Ascaris* sp., *Trichuris trichiura*, *T. vulpis*, *Toxocaris* sp., *Toxocaris leonia*, *Capillaria* sp.
[b] Mixture of 1° and waste activated sludge
[c] Based on microscopic examination of ova only

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collected from eight treatment facilities that practice one- and two-stage anaerobic digestion (LaConde, 1978). In this survey (Tables 36 and 37), 38 percent of the 16 raw sludge samples collected were found to contain viable parasite ova at densities of 50 or less ova per gram dry weight (GDW), and 25 percent of the 16 digested sludge samples were found to contain viable parasite ova at densities of less than 50 to more than 50 per GDW.

Viable ova of the dog hookworm, *Ancylostoma canium*, seeded into digesting sludge, were demonstrated for periods up to 64 days at 20°C, 41 days at 30°C and 36 days at room temperature (Cram, 1943).

Encysted *Trichinella spiralis* larvae seeded into laboratory-scale digesters appear to be far more sensitive to the digestion process than the other nematode species discussed above. Fitzgerald and Prakasam (1978) found that larvae survived only 96 hours of digestion at 35°C (14 days' detention). Daily inoculation of another digester with *Trichinella* cysts resulted in viable larvae being recovered continuously over the course of the experiment.

Additionally, Fox and Fitzgerald (1977) examined anaerobically digested sludges from four treatment plants. Eggs or cysts of eight genera of metazoans and two of protozoans (genera of the coccidia subclass and *Entamoeba coli*) were enumerated as follows:

Taxa	Number per gram
Ascaris lumbricoides	0.11 - 1.2
Toxocara sp.	1.01 - 3.40
Toxocaris leonina	0 - 0.16
Trichuris sp.	0 - 0.23
Cestodes (*Taenia* sp. and *Hymenolepsis* sp.)	0 - 0.33
Coccidia (*Eimeria* sp. and *Isospora* sp.)	0 - 3.72
Entamoeba coli	0 - 0.34

TABLE 36. DENSITY LEVELS OF TOTAL PARASITE OVA BEFORE AND AFTER TWO STAGE ANAEROBIC DIGESTION AT FULL SCALE WWTPs AT MESOPHILIC TEMPERATURES

Densities (ova/gdw)						Detention Time (days)	Temperature (°C)	VS Reduction	Sludge type	Author
Raw			Digested							
Count	N	Organism	Count	N	Organism					
ND[a]	2	-	>50 ND	1 1	Toxacara canis, Ascaris lumbricoides, Coccidian	30 + 90	35	NS[b]	Mixed trickling filter + 1°	Laconde (1978)[c]
<50 <50	1	T. canis	ND <50	1 1	A. lumbricoides, T. canis	20	35	NS	Mixed waste activated + 1°	LaConde (1978)[d]
<50	1	A. lumbricoides coccidian oocyst	ND	1	-	21 + 21-60	35	NS	Mixed trickling filter + 1°	LaConde (1978)[e]
<50	1	T. canis	<50	1	T. canis					
ND	2	-	ND	2	-	10+ 20	35	NS	Mixed waste activated + 1°	LaConde (1978)[f]
ND	2	-	ND	2	-	150+ 100	35	NS	Mixed waste activated + 1°	LaConde (1978)[g]

[a] ND = Not detected
[b] NS = Not specified by author
[c] Macon, Ga. WWTP
[d] Springfield, Mo. WWTP
[e] Hopkinville, Ky. WWTP
[f] Wilmington, Ohio WWTP
[g] Chippewa, Wi. WWTP

TABLE 37. DENSITY LEVELS OF TOTAL PARASITIC OVA BEFORE AND AFTER ANAEROBIC DIGESTION AT FULL SCALE WWTP AT MESOPHILIC TEMPERATURES

Densities (ova/GDW)						Detention Time (days)	Temperature (°C)	VS Reduction	Sludge type	Author
Raw			Digested							
Count	N	Organism	Count	N	Organism					
50	1	Hookworm ova, *A. lumbricoides*	50	1	*A. lumbricoides*	45	35	NS[a]	Mixture of 1° + trickling filter	LaConde (1978)[b]
50	1	*T. canis*	ND	1	-					
ND	2	-	ND	2	-	14	35	NS	Mixture of 1° + trickling filter	LaConde (1978)[c]
ND	2	-	ND	2	-	30	35	NS	Mixture 1° + waste activated sludge	LaConde (1978)[d]

[a] NS = Not specified
[b] Frankfort, In. WWTP
[c] Kendallville, In. WWTP
[d] Columbus, In. WWTP

Discussion and Conclusions

Reductions and Density Levels Achieved. High-rate anaerobic digestion of municipal sludge at 35°C with a detention time of 14 to 15 days produces a 1-to 1.5-log reduction of indicator bacteria (total coliforms, fecal coliforms and fecal streptococci), *Salmonella* sp. and naturally occurring enterovirus.

Treatment facilities from New York State to California achieve reductions in these organisms that result in the density levels presented in Table 38. Reductions in densities of the opportunistic pathogen *Pseudomonas aeruginosa* averaged less than 1 log in data reported at two treatment facilities.

Ova or cysts of parasitic tapeworms, flatworms and roundworms are able to survive the digestion process (with the exception of *Trichinella spiralis*), while parasitic protozoans are reduced to non-detectable levels (Cram, 1943; Reimers *et al.*, 1980).

Virtually no data were reported in the literature on the reduction of indicators, pathogens or viruses in a digester operating at temperatures of less than 20°C. Under batch conditions, seeded poliovirus type 1 (CHAT) were reduced by 0.28 log/day at 4°C and 0.91 log/day at 20°C (Ward and Ashley, 1977; Table 28).

Ova of *Schistosoma japonicum* (Newton *et al.*, 1948) were shown to survive at temperatures of less than 20°C for greater than 60 days (Table 33).

Operating Parameters. Two parameters were considered in evaluating the efficiency of anaerobic digestion in reducing the levels of indicators, pathogenic bacteria, enteric viruses and parasites: detention time and temperature.

Detention time can be viewed in two senses when examining data from full-scale and laboratory studies. Generally, the longer the organism is in the reactor environment, the greater the overall reduction produced. In studies in which solids and organisms enter the digester together, solids detention time is equal to organism detention time. This is usually the case in full-scale studies and is seldom the case in laboratory-scale studies reviewed. In studies in which a digester solids' detention time is kept constant and an inoculum of the organism in question is added, the digester detention time may differ from organism detention time. In such studies, it is the *rate* of organism decline, the reduction achieved per unit time -- that is of interest. Such studies are of value in analyzing the effects of specific operating parameters, such as temperature or set detention time.

The relationship between detention time and reduction of indicator bacteria and *Salmonella* sp. at 3 full-scale plants is illustrated in Figure 5. With some variation, it can be seen that the longer the detention time, the

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TABLE 38. DENSITY LEVELS OF INDICATOR BACTERIA, PATHOGENIC BACTERIA AND ENTEROVIRUS FOLLOWING HIGH RATE ANAEROBIC DIGESTION FOR 14-15 TO 21 DAYS AT 35°C (FULL SCALE TREATMENT PLANT DATA)

Organism	Density Level[a] Per 100 ml	Log Reduction		
		Mean[b]	Range	Table reference
Total Coliform	3×10^7 [c]	2.05	1.78-2.30	D4
Fecal Coliform	2×10^6 [c]	1.84	1.44-2.33	D5, D7
Fecal Streptococcus	9×10^5 [c]	1.48	1.10-1.94	D6, D8
Salmonella sp.	3.7×10^1 [d]	1.63	0.91-2.08	D17
Ps. aeruginosa	6×10^5 [d]	0.58	0.15-1.0	D17
Enterovirus	7.9×10^1 [e]	1.21	1.05-1.36	D25

[a] Arithmetic average of mean (geometric) values
[b] Arithmetic average
[c] Count per 100 ml
[d] Most probable number per 100 ml
[e] Plaque forming units per 100 ml

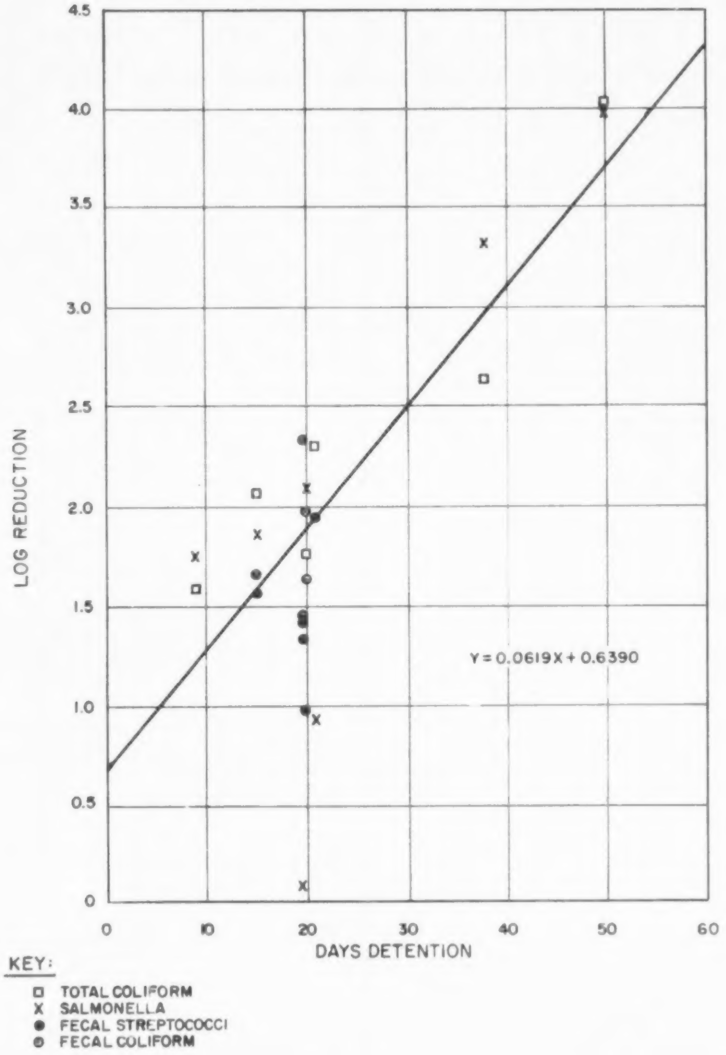

FIGURE 5. REDUCTION OF INDICATOR BACTERIA AND SALMONELLA WITH DETENTION TIME AT 34°-37° C.

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greater reduction achieved. A regression analysis of these data revealed that log reduction of bacteria depended significantly on detention time at the 0.01 probability level.

The response of seeded virus is found to differ from that of bacteria when inoculated into laboratory digesters operated at mesophilic temperatures and at solids retention times ranging from 5 to 20 days. Little difference is seen in the secondary rate of reduction (rate after the first 24 hours) of poliovirus type 1 at 5, 10, or 15 days solids retention time. (Sander *et al*. 1977, shown in Figure 2). Whatever the factors are that contribute to virus die-off, they do not appear to increase or are not enhanced with longer solids retention time. A trend is indicated that at temperatures of less than 34°C, solids retention time plays an increasingly important role in the rate of inactivation of poliovirus. In contrast, seeded *Salmonella typhosa* (McKinney *et al*., 1958) is reduced by 4 logs within 2 days in digesters operated at 20 days solids retention time, compared to a reduction of 2.2 logs in that same time in a digester with a 6 day solids retention time.

Temperature is the primary controller in the die-off of indicator and pathogenic bacteria, enteric viruses and parasites. At 49°C, 1- to 3-log increases over reduction at 35°C were reported by Berg and Berman (1980) and Stern and Farrell (1977), as shown in Figure 6. Similar responses are seen with the blood fluke, *Schistosoma japonicum* (Figure 7). Responses of other parasites (such as the roundworm *Ascaris lumbricoides*) that can encyst, or of spore-forming bacteria, have not been adequately studied in an anaerobic digester with regard to temperature.

Laboratory-Scale vs Full-Scale Studies. An insufficient amount of data is available on bacteria and parasite levels from experiments in which operating parameters have been controlled in a similar-enough fashion to allow for valid comparisons between reductions on a full scale vs. reductions on a laboratory or pilot scale. More data are available on reduction of enterovirus, and they indicate that much greater reductions are seen in laboratory-scale digesters (which generally utilized seeded virus and produced a mean log reduction of about 1 log per day) than at full-scale plants (1-log reduction during a 20-40 day retention). The differences in reduction seen for enteroviruses can be attributed to a number of factors, most of which would also apply to bacteria and parasites.

One major factor is that of short circuiting -- a certain portion of fresh influent sludge being allowed to exit prematurely and, with it, high levels of organisms -- which can occur at full-scale plants. It is possible in this situation that the destruction of viruses (or other organisms) is occurring at full-scale plants at the same rate as in laboratory-scale experiments, but that the residual levels measured in the full-scale plants are those introduced in the previous 24 hours.

In laboratory-scale studies in which bacteria were continuously inoculated (McKinney *et al*., 1958), a 12- to 24-hour period was allotted before the

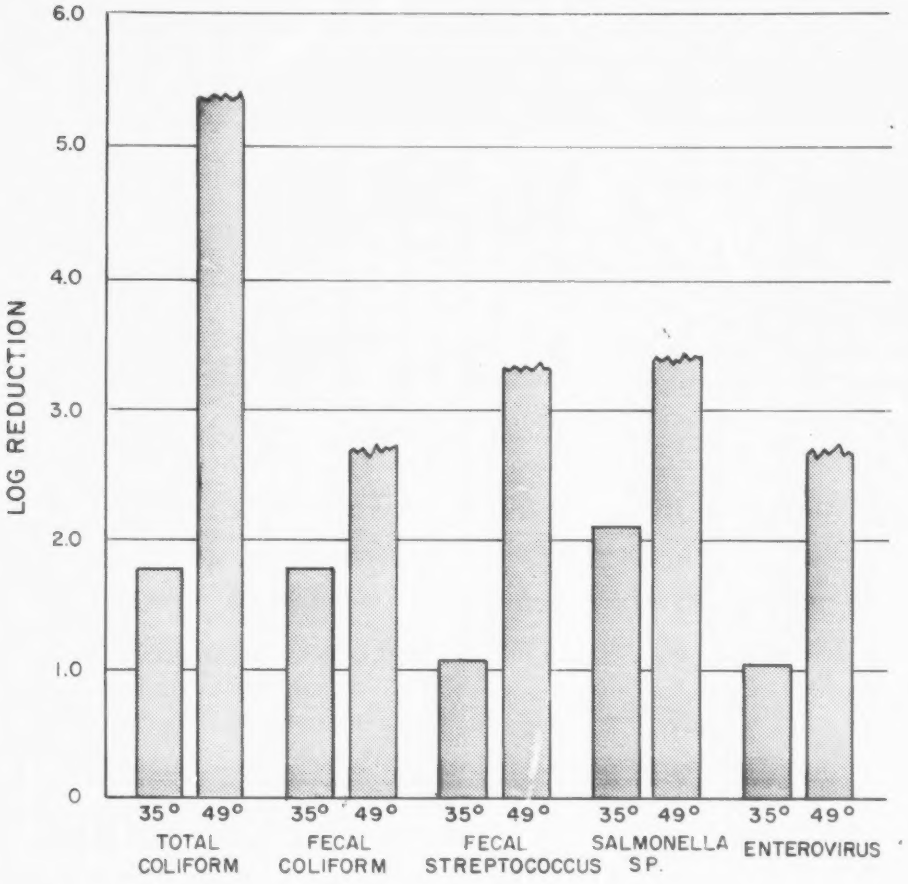

FIGURE 6. REDUCTION OF INDICATOR BACTERIA, PATHOGENIC BACTERIA AND ENTEROVIRUS DURING HIGH-RATE ANAEROBIC DIGESTION *

* BERG AND BERMAN (1980) AND STERN AND FARRELL (1977)

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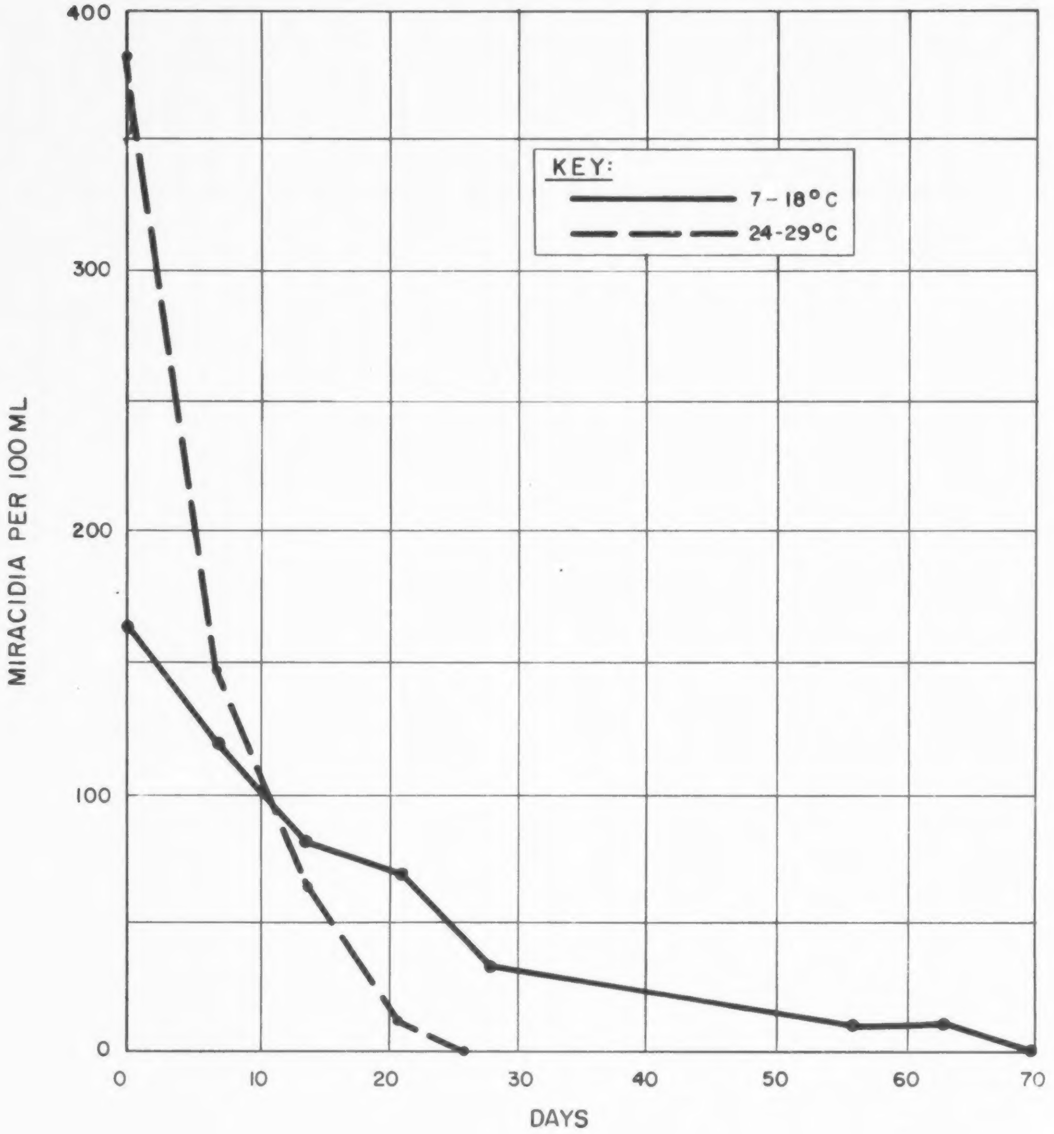

FIGURE 7. REDUCTION OF SCHISTOSOMA JAPONICUM OVA INOCULATED INTO A LABORATORY ANAEROBIC DIGESTER*

* NEWTON et al. (1948) JONES et al. (1947)

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draw-off. Researchers conducting virus studies have uniformly injected a single inoculum of virus into digesting sludge that was subsequently diluted with fresh, unseeded sludge and the die-off monitored.

A second factor that may vary in full-scale plants vs. those of laboratory or pilot scale is temperature. In full-scale plants, temperature may not be constant throughout the reactor. As pointed out previously, temperature is the primary agent in the destruction of most organisms. A difference in reduction of enteroviruses can be seen with even a 3°C difference in temperature (Sanders *et al.*, 1979; Eisenhardt *et al.*, 1977). This is another cause of lower reductions being achieved *during* digestion at full-scale plants. Temperatures in most laboratory-scale studies are controlled by seating the digester in a water bath, assuring continuous contact with a proper temperature.

A final factor that may influence response of organisms is their origin. It is probable that viruses grown on tissue culture are more susceptible to the effects of anaerobic digestion than indigenous virus. This susceptibility has been described for laboratory strains of *E. coli* (Hinesley, 1974) and discussed for ova of *A. lumbricoides* var *suum* (Reimers *et al.*, 1980) originating from uteri of worms vs. those obtained directly from the intestines of swine.

It can be concluded that while laboratory/pilot-scale work is a good indicator of reductions possible under optimum conditions, such studies should be judged in light of the operating conditions obtainable on a plant-by-plant basis.

Relationship of Indicator Bacteria to Pathogens and Enteroviruses. Total coliforms, fecal coliforms and fecal streptococci, being far easier to quantify than pathogenic bacteria and being present in much greater densities than organisms of known health significance--pathogenic bacteria and specific strains of virus--are used to indicate the probable presence of these organisms. The usefulness of these organisms can be evaluated from two aspects: (1) Do density levels of an indicator in sludge maintain a constant relative value to the density of pathogens and virus? and (2) Is the reduction of indicator bacteria during a treatment process similar to the reduction of pathogens and viruses?

In order to be a valid indicator of pathogen/virus density in digested sludge, the indicator bacteria must first maintain a constant relative value to pathogens/viruses in raw sludge. As pointed out by Berg and Berman (1980):

> "Since the number of viruses and indicators in raw sludges are the basis for the numbers of viruses and indicators in digested sludges, the quantitative relationships between them in digested sludge cannot be more quantitative than in raw sludges."

Figures 8 and 9 indicate that neither total coliform bacteria, fecal coliform bacteria nor fecal streptococcus bacteria are good indicators of Salmonella or enteroviruses in raw sludge (mixtures of primary and waste activated sludge). It should be noted that these figures are prepared from data collected at different treatment plants. In these figures, a diagonal line would indicate a constant relative value between the densities of indicator and pathogenic organisms in raw sludge. The fact that the line of best fit in Figures 8 and 9 is vertical or near-vertical demonstrates, unfortunately, that a given count of any indicator type could indicate a wide range in numbers of Salmonella or enteroviruses.

Because pathogens are variably present, dependent on the number of ill people in the discharging population, it would not be expected that Salmonella density would maintain a constant ratio to indicator bacteria density. It could be expected, however, that many of the enteroviruses should be present in a ratio somewhat similar to that of indicators.

The relative usefulness of total and fecal coliform bacteria and fecal streptococci in indicating the reduction of both Salmonella and viruses during high-rate anaerobic digestion are presented in Figures 10 and 11. These figures compare log reduction of Salmonella and viruses to log reduction of indicators. Data were gathered from four reports (a total of six WWTPs) in the case of Salmonella: indicator, and from two reports (two WWTPs) in the case of virus: indicator comparisons.

The diagonal line indicates a 1:1 ratio of Salmonella:virus destruction to indicator destruction. Points above the line indicate a greater reduction of Salmonella:virus than indicator, while those below the line indicate a greater reduction of indicator than Salmonella:virus. In the case of Salmonella/indicator (Figure 10), there is little difference in the relative reductions of pathogen:indicator. In the case of virus indicator, examination of Figure 11 (a-c) reveals that while none of the comparative reductions of the three indicators is perfectly correlated with enterovirus reduction, fecal streptococcus bacteria (11c) are the most conservative indicator.

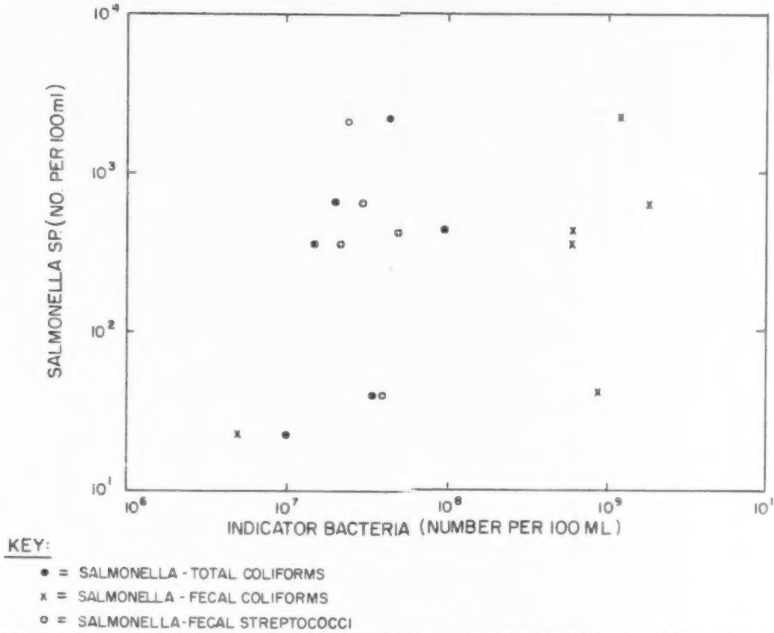

FIGURE 8. RELATIONSHIP BETWEEN NUMBERS OF INDICATOR BACTERIA AND SALMONELLA SP IN RAW SLUDGE.

* JEWELL et al. (1980)

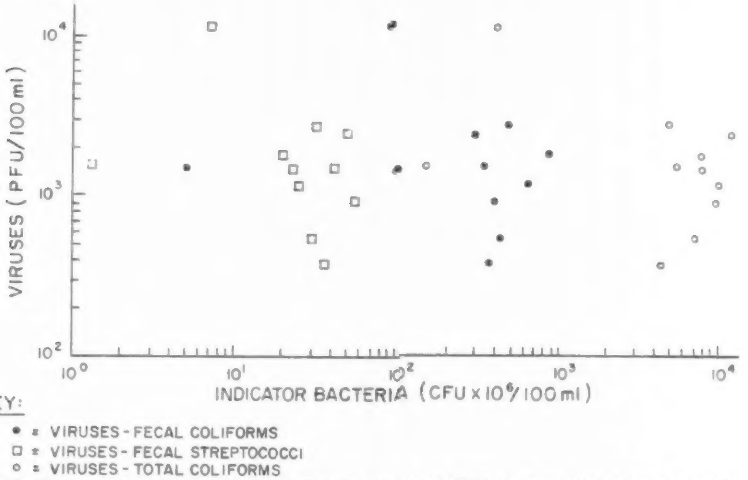

FIGURE 9. RELATIONSHIP BETWEEN NUMBERS OF INDICATOR BACTERIA AND NUMBERS OF VIRUSES IN RAW SLUDGE *

* BERG AND BERMAN (1980)

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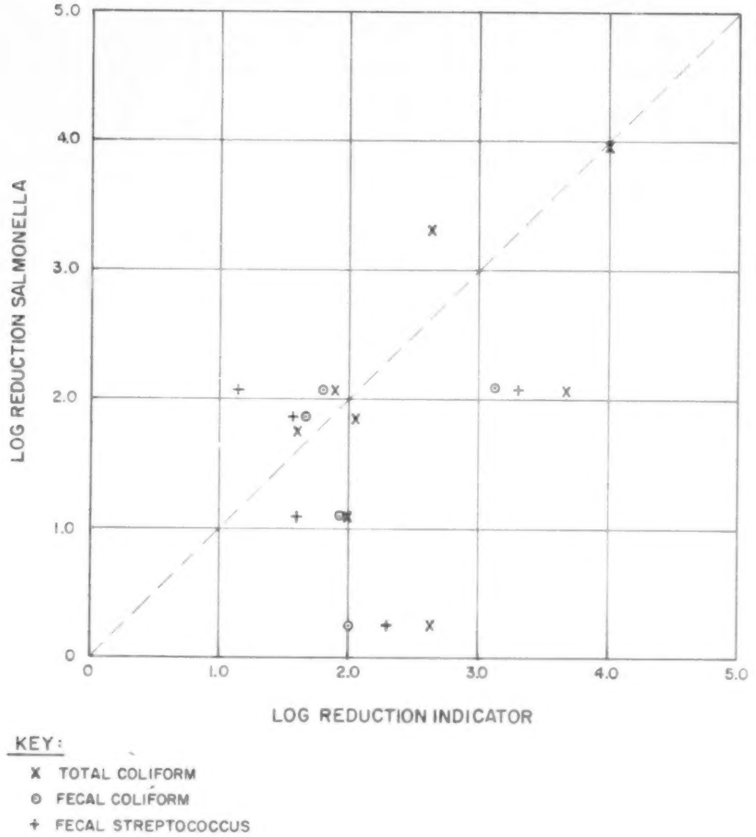

FIGURE 10. REDUCTION OF SALMONELLA VS. REDUCTION OF INDICATOR BACTERIA.*

* STERN AND FARRELL (1977) AND BERG AND BERMAN (1980) - HYPERION TREATMENT PLANT, CITY OF LOS ANGELES, CA.
LUE-HING et al. (1977) - METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO, IL.
JEWELL et al. (1980) - BINGHAMPTON - JOHNSON CITY TREATMENT PLANT, N.Y.
COOKE et al. (1978) - METRO NASHVILLE'S DRY CREEK SEWAGE TREATMENT PLANT
SINKING CREEK NO 2, MURFSBORO, TENN.
LYTLE CREEK PLANT NO. 1, MURFSBORO, TENN.

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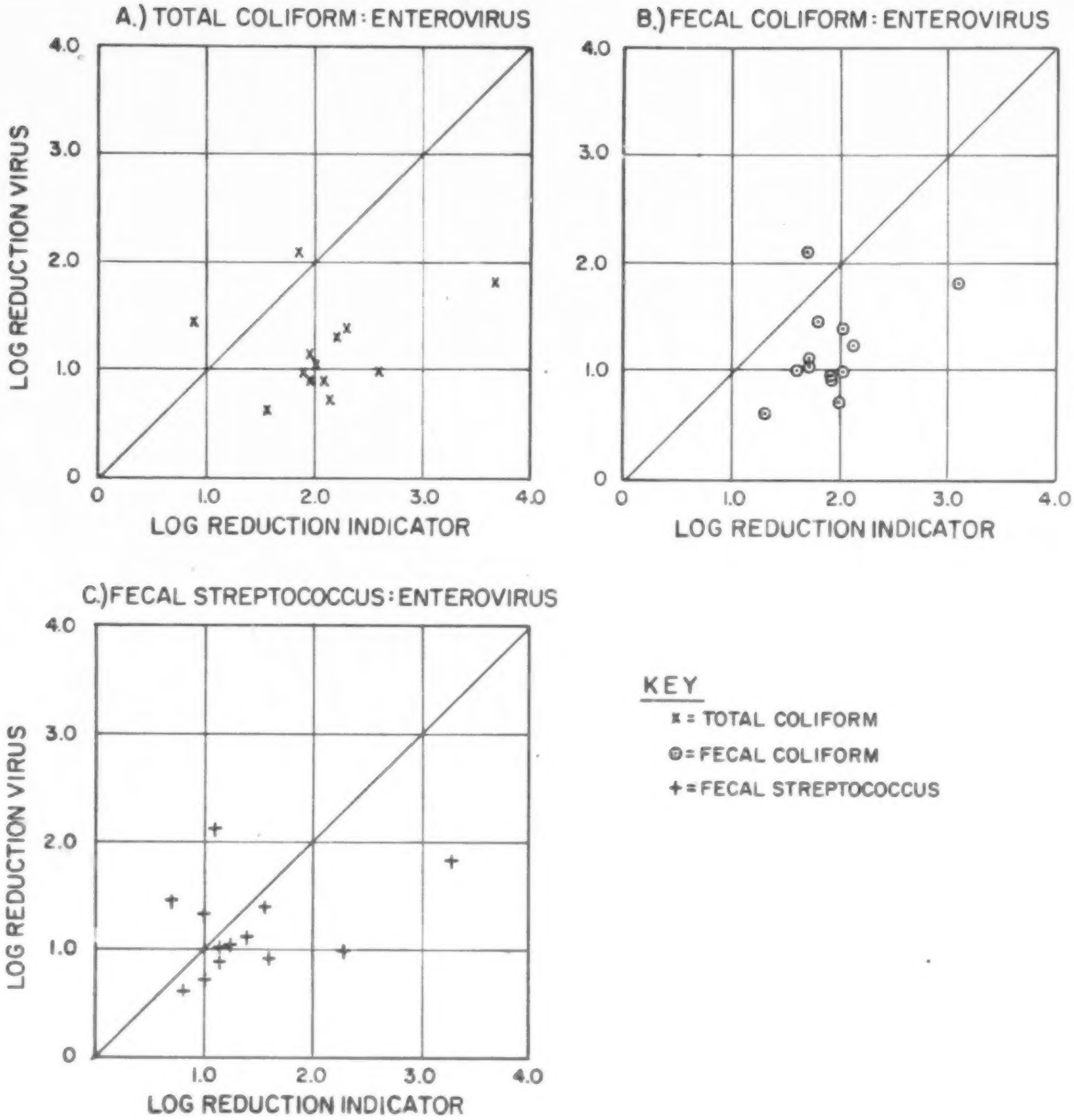

FIGURE 11. REDUCTION OF INDICATOR BACTERIA VS. REDUCTION OF ENTEROVIRUS.*

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* BERG AND BERMAN (1980)
JEWELL et al. (1980)

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AEROBIC DIGESTION

Introduction

Description of Process. In aerobic digestion, wastewater sludge is aerated in tanks. The process provides a biochemical, oxidative means of stabilization.

Operating Parameters. This review covers aerobic digestion in the mesophilic range, to a maximum temperature of 37°C. Aerobic digesters are, in fact, commonly operated at ambient temperatures that rarely surpass 25°C. As defined in EPA's *Criteria*, required sludge detention times in mesophilic aerobic digestion vary inversely with temperature: 60 days at 15°C to 40 days at 20°C. Reduction of volatile solids in this period should be 38 percent or greater. (Thermophilic aerobic digestion, carried out at temperatures of 55 to 60°C, can achieve substantial reductions of pathogens and volatile solids within much shorter detention times, on the order of 10 days. EPA has defined this practice as a Process to Further Reduce Pathogens.)

In actual practice at full-scale installations, detention times for mesophilic aerobic digesters are based solely on volatile solids reduction, and not on effective reduction of indicator or pathogenic organisms. At these installations, detention times in continuous and intermittently-fed digesters generally range from 10 to 20 days.

Date Base Available. A severely limited data base is available on levels of pathogens, viruses and parasites during aerobic digestion in the mesophilic range. A brief description of the literature reviewed on this topic is presented below:

- Bitton *et al*. (1980)--A 10-acre sludge-disposal site at Lake Kanapaha, Florida was monitored for survival of enteroviruses in soils amended with centrifuged, aerobically digested sludge from a wastewater-treatment plant in the City of Gainesville. Additionally, aerobically digested sludge from a Pensacola wastewater-treatment plant was examined for presence of enteroviruses.

- Hess and Breer (1975)--A total of 219 samples of raw, "aerobically stabilized" and anaerobically digested sludges from 44 wastewater-treatment plants were examined for *Escherichia coli* and *Salmonella* spp. (It is assumed from the context of the paper that "aerobically stabilized" is equivalent to "aerobically digested"; no further description or operating parameters are offered, however.)

- Hurst *et al*. (1978)--Enteroviruses in sludge samples from full-scale wastewater-treatment plants were enumerated in a paper, the primary purpose of which was to present a methodology developed for detecting viruses in sludge. One sample of thickened, aerobically digested, and centrifuged sludge was among those examined.

- LaConde *et al.* (1978a, b)--As part of an EPA-sponsored environmental assessment on application of sludges to agricultural land, these researchers analyzed two samples of aerobically digested sludge from a plant in Las Virgenes, California for fecal coliform and fecal streptococcus bacteria, *Salmonella* spp. and *Shigella* spp., and parasites.

- Smith and Hathaway (via Farrell, 1979)--Pilot-scale batch aerobic digesters were established in order to compare air and oxygen aeration for digesters operating at 22°C. Percent reductions of fecal coliforms, *Pseudomonas aeruginosa* and *Salmonella* spp. were determined over 9 and 15 days in two experimental runs, but no actual density values are reported.

- Reimers *et al.* (1980) -- Experiments were conducted in 20-liter laboratory units at temperatures of 28°C, 35°C, 45°C, 55°C at a 10-day hydraulic detention time. Known numbers of *Ascaris* and *Toxocara* ova were added at the beginning of the test. Each day, one-tenth of the contents was removed, and an equal amount of raw secondary sludge was added. Corrections were made in the calculations to allow for the dilution by the raw sludge. The *Ascaris* tests used eggs from the uteri of worms and from the intestinal content of swine. Uterus-derived eggs were substantially reduced after two days, while the eggs from the swine intestines remained viable. Data from the swine-intestine eggs were thought to mirror more accurately the actual conditions in full-scale plants.

In addition, Danielsson (1977) incubated *Salmonella*-inoculated sludge aerobically for 24 days, with 1 hr of stirring per day. The author considered that this treatment simulated aerobic digestion; the technique in fact differs significantly from actual practice of aerobic digestion at full-scale installations, so we have excluded the results from this review.

Results

Indicator Bacteria. Hess and Breer (1975) found that raw-sludge samples had a geometric mean density of 7.2 x 10^7 *E. coli* per 100 ml, while "aerobically stabilized" samples had a mean density of 2.0 x 10^6 *E. coli* per 100 ml (Table 39). The data imply that a better than 1-log reduction in densities of *E. coli* is possible through aerobic stabilization at full-scale plants, but, as process operating parameters are not reported in the paper, the data are of limited usefulness.

LaConde *et al.* (1978a, b) counted fecal coliform and fecal streptococcus bacteria in two samples of combined primary and waste activated sludge from WWTPs in Las Virgenes, California, and compared original densities of these indicators with those found in a single sample of aerobically-digested sludge from the same plant (20 days detention time). Results are presented in Table 40. The reported original densities of fecal coliform bacteria in this study are out-of-line with the densities reported by most researchers (see Section 2), and the small sample size precludes use of these data in analysis.

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TABLE 39

DENSITIES OF *E. COLI* IN RAW AND AEROBICALLY-STABILIZED SLUDGES*

Sample	Density (no. per 100 ml)	
	Range	Mean
Raw Sludge (n=42)	10^6 - 10^9	7.2×10^7
Aerobically-Stabilized Sludge (n=25)	10^3 - 10^7	2.0×10^6

*From Hess and Breer (1975)

TABLE 40

DENSITIES OF FECAL COLIFORM BACTERIA IN RAW AND AEROBICALLY-DIGESTED SLUDGE*

Sample	Density (MPN/g dry weight)	
	Fecal Coliform Bacteria	Fecal Streptococcus Bacteria
Raw Sludge (n=2)		
Range	$0 - 9.3 \times 10^2$	$8.0 \times 10^4 - 3.1 \times 10^8$
Mean	3.1×10^1	5.0×10^6
Aerobically-Digested Sludge (n=1)	2.7×10^6	2.7×10^8

*From data of LaConde *et al.* (1978a, b)

Smith and Hathaway (via Farrell, 1979) attempted to identify the relationship between detention time and microorganisms kill in batch digesters operated under laboratory conditions. Log reductions in the density of fecal coliform bacteria are shown in Figure 12; actual values of densities were not reported. These indicator bacteria responded erratically to the digester environment (assuming the environment was, in fact, kept constant). Reductions of 1 log or greater in magnitude are seen in two instances, after 6 to 7 days detention time, but, in one run, no significant reduction is seen throughout a 15-day detention period.

Pathogenic Bacteria. Under the same conditions as are described above for their work on indicator bacteria, Hess and Breer (1975) enumerated *Salmonella* spp. in samples of raw sludge and of sludge that had been "aerobically stabilized" at full-scale installations. Their findings are presented in Table 41. Again, the authors provide no indication of the operating parameters in the "aerobic-stabilization" systems from which samples were obtained.

LaConde *et al*. (1978) did not detect either *Salmonella* spp. or *Shigella* spp. in their tests of raw (two samples) and aerobically digested (one sample) sludge from treatment facilities in Las Virgenes, California. Smith and Hathaway (via Farrell, 1979) compared air and oxygen aeration techniques and detention times of 9 and 15 days for their effects on two genera of pathogenic bacteria, *Pseudomonas aeruginosa* and *Salmonella* spp. (Figure 13). The response of *Ps. aeruginosa* is seen to be erratic, and at no point is a reduction of even 1 log in magnitude achieved. *Salmonella* spp., on the other hand, appear to be more sensitive to the conditions of aerobic digestion.

Viruses. Bitton *et al*. (1980) analyzed for enteroviruses in samples of raw waste activated sludge, sludge aerobically digested for 90 days, and sludge aerobically digested for 180 days and dewatered by centrifuge. In addition, the researchers analyzed samples of sludge that had been aerobically digested for 30 days at a Pensacola, Florida, wastewater-treatment plant (levels prior to digestion were not quantified). Results are presented in Table 42.

In one additional study, Hurst *et al*. (1978) examined one sample of waste activated sludge that had been thickened, aerobically digested, and dewatered by a centrifuge unit; they recorded 3.84 plaque-forming units (PFU) per gram dry weight in the raw sludge, 3.35 PFU/g dry weight in the treated sludge. Parameters of digester operation were not reported.

Parasites. Reimers *et al*. (1980) found that *Ascaris* eggs from the intestinal contents of swine are not reduced by aerobic digestion at temperatures of up to 35°C, for detention periods of up to 10 days in intermittently fed laboratory units. In that period, *Toxocara* eggs decreased by over one log at 35°C, with most of the reduction occurring during the first two days. At 45°C, reduction of *Toxocara* was about 0.52 logs.

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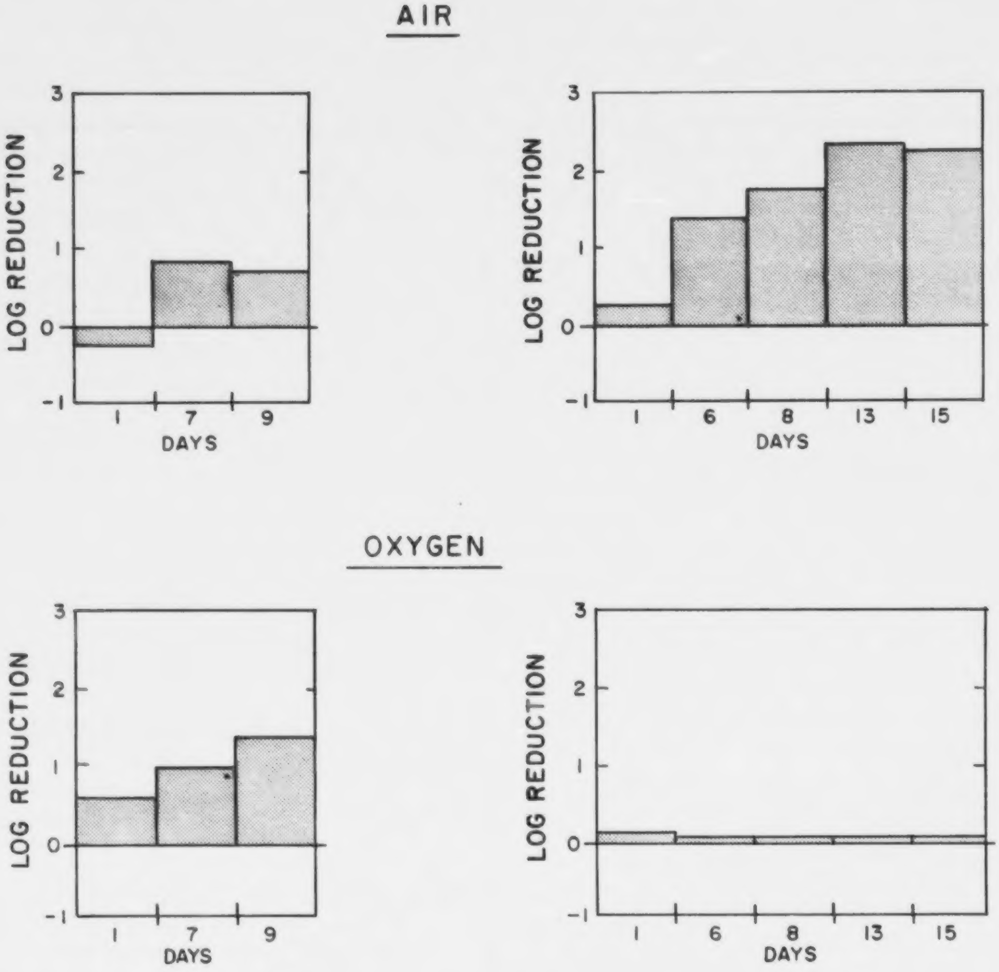

FIGURE 12. EFFECTS OF AERATION TECHNIQUE AND DETENTION TIME ON DENSITIES OF FECAL COLIFORM BACTERIA IN AEROBICALLY-DIGESTED SLUDGE.*

* FROM DATA OF SMITH AND HATHAWAY, VIA FARRELL (1979)

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TABLE 41

DENSITIES OF SALMONELLA SPP. IN RAW AND AEROBICALLY-STABILIZED SLUDGES*

Sample	Density (no. per 100 ml)	
	Range	Mean
Raw Sludge (n=56)	10^{-1} - 10^{6}	4×10^{3}
Aerobically Stabilized Sludge (n=28)	10^{-1} - 10^{6}	3×10^{2}

*From data of Hess and Breer (1975)

TABLE 42

DENSITIES OF ENTEROVIRUSES IN RAW AND DIGESTED SLUDGES*

Sample	Density ($TCID_{50}$)[a]
Gainesville, Florida WWTP	
o Raw Sludge (n=2)	11 - 25
o Digested Sludge (n=2) 90-days' detention	0.3 - 1.2
o Digested Sludge (n=3) 180-days' detention and centrifuge dewatering	ND [b]
Pensacola, Florida WWTP	
o Digested Sludge (n=3) 30-days' detention	
Range	14 - 260
Mean	53

* From data of Bitton *et al.* (1980)

[a] Tissue culture infectious dose for 50-percent response per gram dry weight

[b] ND= Not detected

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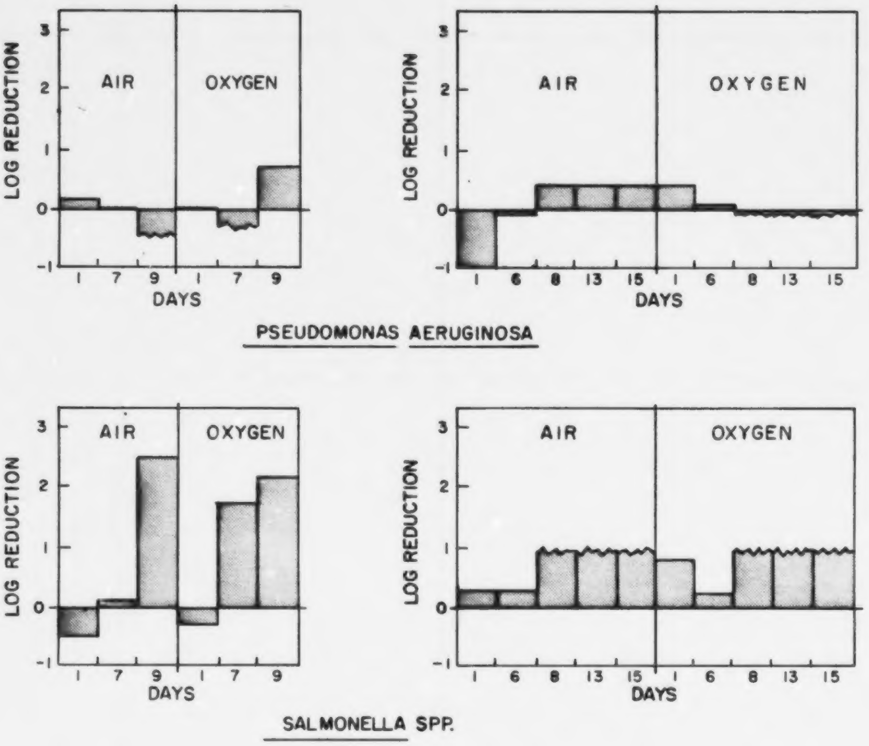

FIGURE 13. EFFECTS OF AERATION TECHNIQUE AND DETENTION TIME ON DENSITIES ON DENSITIES OF PSEUDOMONAS AERUGINOSA AND SALMONELLA SPP. IN AEROBICALLY-DIGESTED SLUDGE. *

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* FROM DATA OF SMITH AND HATHAWAY, VIA FARRELL (1979)

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In batch digesters at 28°C, the number of viable *Ascaris* eggs fell by 0.51 log in 10 days, 0.68 log in 15 days, and 1.40 logs in 20 days. Reimers *et al*. (1980) speculate that the improved reductions with batch processing might be due to a change in the microbial population as a result of the reduction in carbon source.

Discussion

If mesophilic aerobic digestion achieves reductions in densities of indicator and pathogenic bacteria, it does so largely by removing bacterial food sources. Analysis of the effectiveness of this sludge-stabilization process in killing pathogens should, therefore, be related to reduction in volatile solids. No such analytical work has been done. As a result, the available data provide no reliable measure of bacterial kill in aerobic digestion.

Some organisms--*E. coli* and *Salmonella*--are reported to have been reduced in density by 1 log or more after several days of detention (Hess and Breer, 1975; Smith and Hathaway, via Farrell, 1979). In the former reference, however, no conditions or parameters of operation are reported for the aerobic-stabilization process. It is impossible to tell if the relatively large number of samples were obtained from installations that were practicing aerobic digestion as it is commonly practiced in the United States. In the latter reference, laboratory-scale work was conducted in batch operation. The fact that observed reductions are of low orders of magnitude in batch operation implies that at full-scale installations, where continuous or intermittent feed of raw sludge is the rule, reductions in densities of microorganisms will be insignificant unless sludge is held for extended residence periods.

Bitton *et al*. (1980) found that aerobic digestion of waste activated sludge for 90 days achieved a 1-log reduction in enterovirus density. The dearth of information on survival of enteroviruses in sludge treated by aerobic digestion has prompted the U.S. Environmental Protection Agency to fund an additional study by the same researcher (Dr. Gabriel Bitton, Department of Environmental Engineering Sciences, University of Florida, Gainesville).

From only one available study (Reimers *et al*., 1980), aerobic digestion at mesophilic temperatures appears ineffective in decreasing the number of viable *Ascaris* in intermittently fed units. Reductions of 0.5 log can be achieved in 10 days at 28°C in batch units, however.

Toxocara eggs decrease by about 1 log in about 2 days at 35°C in continuous-flow units with a 10-day detention time. An identical digester at 45°C provided only a 0.52 log reduction in 10 days.

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MESOPHILIC COMPOSTING

Introduction

Description of Process. Composting is a natural, aerobic, microbiological process by which organic matter is decomposed into a well-stabilized product--humus, or humus-forming substances. When applied to stabilization of sewage sludges, the process commonly involves combining dewatered sludge cake with a bulking agent (such as woodchips, dry compost, or shredded municipal refuse) to between 40 and 70 percent solids, then shaping the mass into piles, beds, or windrows. During the next several days, depending on ambient conditions, the compost mass will go through four basic stages (Gray, 1971):

(1) Mesophilic (ambient to 40°C)

(2) Thermophilic (45° to 65°C)

(3) Cooling, and

(4) Maturing

The compost mass is usually broken down after its initial cooling and is allowed to mature, or "cure", in stockpiles.

The three principal systems that have been used to compost sewage sludge are open-window, forced-aeration, and closed composting systems:

a. Windrow--In a windrow system, the combined sludge and bulking agent is shaped into long piles (windrows) of relatively short height (90 cm to 150 cm). The windrows are periodically turned, sometimes daily, in order to maintain aerobic conditions in the windrows. The open-windrow composting process generally can be completed in 6 to 10 weeks.

   One of the largest windrow systems in the U.S. is located at the Joint Water Pollution Control Plant of the Los Angeles County Sanitation Districts (LACSD). The LACSD windrows are typically 1.5 m high, 3.6 m wide at the base, and 225 m long.

b. Forced aeration--Forced-aeration composting by the static-pile method has been developed since 1974 by the U.S. Department of Agriculture (USDA) at Beltsville, Maryland. A mixture of sludge cake and woodchips is formed into piles about 3.6 m high for a period of 21 to 28 days. During this time, air is blown or pulled through the pile by means of blowers and plastic pipe located at the base of the pile. The compost piles are then broken down, and the compost is cured for 30 days in new stockpiles, after which compost and woodchips are mechanically separated. Normally about 75 percent of the woodchips are recovered for reuse.

c. Closed composting system--Closed or mechanical composting systems involve mixing and aerating the sewage-sludge/bulking-agent mass in a rotating drum, or in moving elevators. Under these conditions, uniformly high temperatures can be achieved, and the composting mass is relatively unaffected by ambient temperature and precipitation. The composting period is generally of two to three weeks duration, with temperatures reaching 70°C.

d. Deep-pile bin--This is an experimental composting mode that has been attempted at Los Angeles County Sanitation Districts' Joint Water Pollution Control Plant in Carson, California. The technique utilizes bins of dimensions 3 m by 3 m by 3 m with an aeration system installed in the bin floor. In the tests conducted by Iacoboni and coworkers, the bin contents were mechanically turned on the third and sixteenth day of the compost cycle. Dewatered centrifuge cake and recycled composted was blended to a 45 percent solids content and composted in this study.

As defined by EPA in 40 CFR 257, composting for at least five days by any of the above methods constitutes an acceptable process to significantly reduce pathogens in sludge, provided that:

(1) a minimum temperature of 40°C within the compost mass is maintained throughout the composting period, and

(2) temperatures of greater than 55°C are attained for at least four hours of the composting period.

For the purpose of this report, these procedures are taken to define "mesophilic composting".

Operating Parameters. High temperatures generated by microbial activity in the composting process can inactivate or destroy many microorganisms present in sludge. Within the protocol for mesophilic composting defined above, the temperatures attained are not instantly lethal to most indicator and pathogenic microorganisms of concern; the effectiveness of the process depends, therefore, not on temperature alone but rather on maintaining the moderately high temperatures throughout the composting mass over a set period of time.

Whenever high temperatures are not uniformly attained, subsequent mixing of the compost mass can cause bacterial populations from low-temperature zones to reinoculate areas where bacteria had been inactivated by higher temperatures.

Factors other than temperature may contribute to control of microorganism densities in composting, but the literature reviewed for this report focuses almost exclusively on heat inactivation.

Data Base Available. An extensive amount of research has been completed on the effectiveness of composting in reducing densities of indicator and pathogenic microorganisms. Unfortunately, little of it can be considered directly pertinent to the "mesophilic composting" concept incorporated in 40 CFR 257.

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Most researchers cited below have worked at full-scale composting facilities in attempts to optimize open-windrow, forced-aeration, and closed processes that are normally expected to operate within thermophilic temperature ranges for much of their composting cycles. They have, therefore, operated systems at significantly higher temperatures and over much longer periods of time than have been defined as minimum operating criteria for mesophilic composting.

For the purpose of this report, we have used data mainly from facilities where temperatures have stayed within the mesophilic range for all, or at least an early stage, of the composting run. Where compost cycles extended over long periods, we have examined the information from the early stages in order to correlate the observations with the 5-day period delineated in 40 CFR 257.

Additional information relating to specific lethal time/temperature relationships for different bacteria and viruses is available through laboratory-scale studies which primarily utilized seeded organisms. The following is a brief synopsis of articles and reports used in this review:

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- Brandon (1978). In this study, composted sludge obtained from the Beltsville, Maryland composting operation was seeded with ova from the uteri of *Ascaris lumbricoides* var *suum*. The inoculated sludge was then held at 45°C, 50°C, and 55°C for up to 7 hr.

- Brannen *et al*. (1975). In order to determine temperature sensitivity, ova from the uteri of *A. lumbricoides* were suspended in sludge supernatant and incubated for up to 60 min at 47°C, 51°C, and 55°C.

- Burge *et al*. (1978). A review of literature and a review of data from the Beltsville, Maryland composting operation were used to develop and compare D values (time for 90% reduction in numbers) for bacteria, viruses, and parasite ova.

- Cooper and Golueke (1978). In these experiments, dewatered (vacuum filtered) digested sludge was mixed with municipal refuse and composted for up to 42 days. In the "large scale" phase of the study, windrows measuring 1.5 m x 1.8 m x 3.0 m were used and turned 1 and 3 times per week. The "small scale" study was conducted in a bin measuring 1.22 m on a side. Naturally occurring total and fecal coliform bacteria, fecal streptococci, and coliphage, as well as seeded poliovirus, type 1, strain LSc were enumerated.

- Cramer and Burge (1975). Digested sludge from the Blue Plains WWTP in Washington, DC was seeded with f2 bacteriophage prior to vacuum filtration. The seeded dewatered sludge was trucked to the Beltsville composting facility, where it was mixed with wood chips (1:3) and composted in a windrow system for up to 70 days. Windrows prepared in the spring, summer, winter, and fall averaged temperatures (sampled at three points within the pile) ranging from

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22°C to 68°C. Following windrow composting, the stabilized sludge was stock-piled for up to 4 weeks.

- Epstein et al. (1976). In this study, the efficiency of forced aeration composting of three sludge types at Beltsville were compared. A combination of primary and secondary undigested sludge; 75 percent raw and 25 anaerobically digested sludge; and anaerobically digested sludge were used. Survival of total and fecal coliform bacteria, and Salmonella after 14 to 21 days of composting was examined.

- Gaby (1975). This study was conducted at the Johnson City open windrow composting plant. A mixture of municipal refuse and primary dewatered sludge was stabilized in windrows measuring 1.2-1.5 m x 2.1-3.0 m x 45 m. The windrows were turned every "several days" for approximately 49 days. Naturally-occurring total and fecal coliform bacteria, and inserted Staphylococcus aureus and Shigella sonnei (in nutrient broth ampules), were enumerated.

- Horvath (1977). This paper reports on the windrow phase of composting operations at the Joint Water Pollution Control Plant, (LACSD), Carson, California, also reported by Iacoboni and coworkers. Windrow systems were constructed using a mixture of centrifuge cake and dry composted sludge in an approximate ratio of 3 cake:1 compost. The piles were turned once a day and sampled for total and fecal coliform bacteria, Salmonella sp. and ova of Ascaris lumbricoides.

- Iacoboni (November 1977). Iacoboni and LeBrun (1978), Iacoboni and Livingston (1977) and (1979). The deep-bin, forced-aeration, and windrow composting systems practiced at the Joint Water Pollution Control Plant, Carson, California has been described in the introduction to this section. Levels of total and fecal coliform bacteria, fecal streptococci, Aspergillus, and viable Ascaris ova were monitored throughout the compost piles.

- Krige (1964). Mature compost collected from various treatment plants in South Africa was examined for viable ova of Ascaris lumbricoides. The age of the compost ranged from 4 weeks to 12 months, and the maximum temperature achieved was only intermittantly recorded.

- Krogstad and Gudding (1975). A 1:1 mixture of municipal refuse and sludge was composted in a forced aeration mechanical reactor for up to 7 days in this study. Survival of inserted and naturally occurring Salmonella typhimurium, Serratia marcescens, and Bacillus cereus were compared, and levels of naturally occurring Escherichia coli monitored.

- Millner et al. (1977). The survival of Aspergillus fumigatus during the Beltsville forced-aeration, static-pile composting operation was monitored in this study. Samples of dewatered (vacuum filtered) sludge, new and composted wood chips, mature compost from three temperature regimes within the piles (less than 40°C, 40°-60°C, greater than 60°C) and stockpiled compost (1 week to 6 months in age) were examined.

- Morgan and McDonald (1969). In this study, Mycobacterium tuberculosis var hominis was inserted into windrow compost piles. The piles were turned every 3 days for 49 days.
- Ward and Ashley (1978). In this laboratory-scale study, raw, anaerobically digested, dewatered (by evaporation) and composted (obtained from Beltsville) sludge was seeded with poliovirus type I (CHAT) and reovirus type 3 (Dearing). The survival of the seeded virus was compared at temperatures ranging from 39°C to 51°C.
- Ward and Brandon (1977). Composted sludge obtained from the Beltsville composting operation was sterilized and inoculated with Salmonella enteritidis serotype montevideo, total coliform and fecal streptococcus bacteria. The inoculated sludge was then held at temperatures ranging, from 50°C to 67°C for up to 50 min.
- Wiley and Westerberg (1969). Candida albicans and ova from the uteri of Ascaris lumbricoides were inoculated into a mechanical composter unit in this laboratory-scale study. The unit utilized dewatered primary sludge which was mixed with augers and force-aerated for a period of 5 days. Temperatures varied from 60°C to 76°C.

Results

Indicator Bacteria. Samples of compost at 50 to 60 percent solids concentration were inoculated by Ward and Brandon (1977) with total coliform bacteria to densities of "typically" 1×10^6 to 1×10^8 per GDW; the samples were then studied for temperature/time relationships in inactivation of the bacteria. Up to 30 minutes exposure at 50°C had no appreciable effect on the organisms. At 52°C, a 1-log reduction was observed after 8 minutes exposure; at 55°C, reductions increased from 1.4 logs to 2.5 logs over 6 to 30 minutes. Coliform bacteria reportedly undergo a 1-log reduction in density within 2 minutes at 55°C, under laboratory conditions (Sivinski, 1978).

At full-scale installations, densities of total coliform bacteria have been studied in open-windrow, forced-aeration, and deep-pile bin composting. Figure 14 shows results obtained by Horvath (1977) for concentrations of total coliform in the interior of windrows turned daily. Also shown in this figure is a record of temperatures achieved during the cycle. A temperature of 40°C was reached after about 4 days of composting. A temperature of 55°C was reached at about 12 days. By the 12th day, the numbers of total coliform bacteria had decreased by about 3.3 logs. These values are for concentrations in the interior of the piles. Samples taken on the surface of the piles might have higher concentrations of bacteria. This is indicated in work by Gaby (1975) who composted solid wastes and liquid sludge. Higher

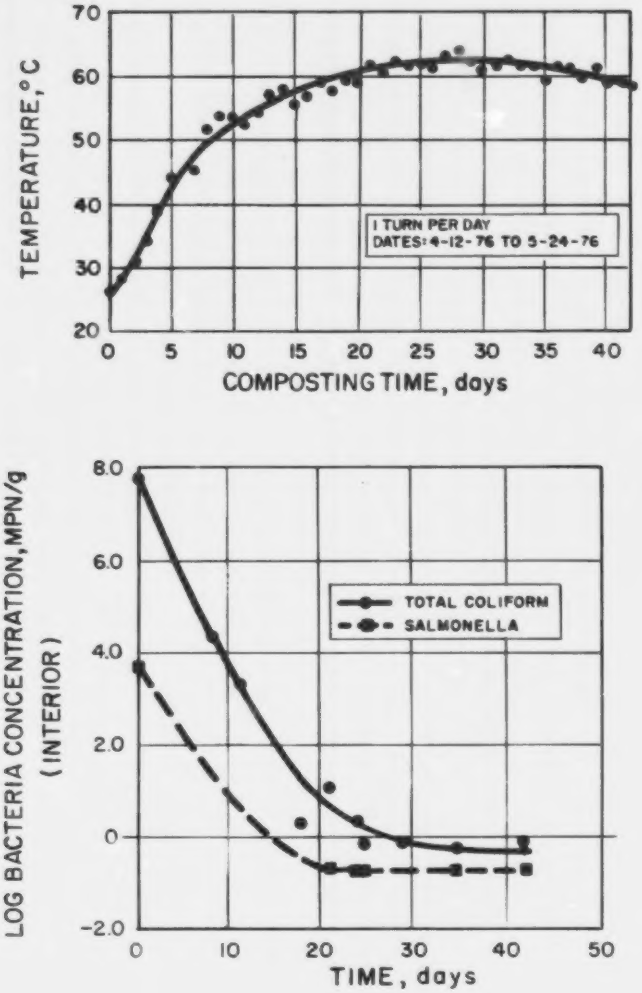

FIGURE 14. REDUCTION OF TOTAL COLIFORM BACTERIA AND SALMONELLA SP. AND CORRESPONDING TEMPERATURES ACHIEVED DURING OPEN WINDROW COMPOSTING. *

* HORVATH (1977)

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bacterial counts were recorded near the surface and near the bottom of windrows in this study.

In Table 43, showing densities of total coliform bacteria in forced-aeration composting systems, two points reported by Iacoboni (November 1977) are of particular interest because they reflect what might occur under the minimum conditions of mesophilic composting. At 4 days duration and internal pile temperatures of 42° to 45°C, Iacoboni observed a 4.7-log reduction in total coliform bacteria to a final density of 1.6×10^3 MPN/GDW. In the top 30 cm of a pile, where maximum temperatures reached 52°C, a 3.1-log reduction was observed.

Table 44 shows densities of total coliform bacteria in deep-bin composting systems. The process is seen generally to effect 3- to 7-log reductions in these bacteria, when operated at mesophilic temperatures over 2 to 3 weeks. Some poorer reductions (Iacoboni, 1977; Iacoboni and LeBrun, June 1978) can be attributed to the failure of the deep-pile system to achieve uniformly high temperatures in the mesophilic range within 9 to 10 days of start-up.

Fecal coliform bacteria have been studied as indicators in static-pile and deep-bin composting installations. Densities of these bacteria have been reported at different temperatures and composting periods, as summarized in Tables 45 and 46. Fecal coliform levels in open windrow composting installations have not been reported in the literature.

Uniformly good reductions of fecal coliform bacteria have been reported in forced-aeration composting systems (Table 45) during mesophilic composting over 25 to 28 days. (Data from shorter periods were not reported.) Epstein et al. (1976) found somewhat higher densities in the toe of the pile than at 40-cm depth in the center, as was also the case for total coliform bacteria.

Several studies of deep-pile bin composting at LACSD's Joint Water Pollution Control Plant in California have shown significant reductions of fecal coliform bacteria over 9 to 24 days of active composting at moderate temperatures. As with total coliform bacteria, poor reductions of fecal coliform bacteria occurred at greater depth in these bins, where temperatures remained low during the first 9 days (Table 46, last row).

A third type of indicator bacteria, fecal streptococcus bacteria, has been studied in deep-pile bin composting (Iacaboni, November 1977). At temperatures between 36 and 58°C, composting was reported to reduce densities of these bacteria by well over six orders of magnitude. It should be noted that the 6.5-log reduction observed after 9 days of deep-pile bin composting is offset by the fact that densities after 26 days of composting at these and higher temperatures were as high as 4×10^3 MPN/GDW--a final log reduction of only 2.9. The bacteria apparently were reactivated during the full compost cycle.

In general, fecal streptococcus bacteria resist the thermal conditions of mesophilic composting better than do the total and fecal coliform bacteria. In laboratory studies, Ward and Brandon (1977) seeded fecal streptococcus

TABLE 43. DENSITIES OF TOTAL COLIFORM BACTERIA IN FORCED-AIR COMPOSTING SYSTEMS

Sampling Point	Temperature (°C)	Duration (days)	Initial Density (MPN/GDW)	Final Density (MPN/GDW)	Log Reduction	Reference
Toe	15-46°	28	3.9×10^{8}	2.8×10^{3}	5.1	Epstein *et al.* (1976)
Top, middle, bottom	26-48°	25	7.7×10^{7}	$\leq 1.0 \times 10^{1}$	≥ 6.9	Iacoboni and LeBrun (May 1978)
Internal	42-45°	4	8.2×10^{7}	1.6×10^{3}	4.7	Iacoboni (November 1977)
Top 30-cm	52° (Max.)	4	8.2×10^{7}	6.1×10^{4}	3.1	Iacoboni (November 1977)
Toe	42-58°	28	5.8×10^{7}	4.1×10^{5}	2.2	Epstein *et al.* (1976)
40-cm depth, center	15-60°	28	3.9×10^{8}	4.3×10^{1}	7.0	Epstein *et al.* (1976)
40-cm depth, center	28-60°	28	3.9×10^{8}	5.0×10^{0}	7.9	Epstein *et al.* (1976)
Toe	48-70°	28	3.9×10^{8}	2.7×10^{5}	3.2	Epstein *et al.* (1976)

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TABLE 44. DENSITIES OF TOTAL COLIFORM BACTERIA IN DEEP-BIN COMPOSTING SYSTEMS

Temperature (°C)	Duration (days)	Initial Density (MPN/GDW)	Final Density (MPN/GDW)	Log Reduction	Reference
36-43°	17	>6.6x10^6	3.1x10^3	>3.3	Iacoboni and Livingston (1977)
32-52°	10	6.2x10^7	9.6x10^4- 4.3x10^5	2.2-2.8	Iacoboni (December (1977)
34° average 58° maximum	24	9.95x10^7	4.67x10^0 (30-cm) 1.97x10^3 (150-cm)	7.3 4.7	Iacoboni and LeBrun (May 1978)
36-58°	9	2.9x10^7	2.3x10^1 (120-cm)	6.1	Iacoboni (1977)
43-59°	9	3.1x10^7	1.0x10^2 (30-cm) 1.0x10^2 (150-cm) 1.0x10^7 (bottom)	5.5 5.5 0.5	Iacoboni and LeBrun (June 1978)

TABLE 45. DENSITIES OF FECAL COLIFORM BACTERIA IN FORCED-AIR COMPOSTING SYSTEMS

Sampling Point	Temperature (°C)	Duration (days)	Initial Density (MPN/GDW)	Final Density (MPN/GDW)	Log Reduction	Reference
Toe	15-46°	28	1.6x10^7	2.3x10^1	5.8	Epstein *et al.* (1976)
Top, middle, bottom	26-48°	25	5.1x10^6	1.0x10^1	5.7	Iacoboni and LeBrun (May 1978)
Toe	42-58°	28	2.6x10^6	2.9x10^1	5.0	Epstein *et al.* (1976)
40-cm depth	15-60°	28	1.6x10^7	<8.0x10^0	6.3	Epstein *et al.* (1976)
40-cm depth	28-60°	28	5.5x10^7	2.5x10^0	7.3	Epstein *et al.* (1976)
Toe	48-70°	28	5.5x10^7	3.4x10^3	4.2	Epstein *et al.* (1976)

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TABLE 46. DENSITIES OF FECAL COLIFORM BACTERIA IN DEEP-PILE BIN COMPOSTING SYSTEMS

Sampling Point	Temperature (°C)	Duration (days)	Initial Density (MPN/gdw)	Final Density (MPN/gdw)	Log Reduction	Reference
NS*	36-43°	17 composting, 14 storage	>6.6x10^6	6.4x10^2	>4.0	Iacoboni and Livingston (1977)
<30-cm depth	28-47°	24	2.5x10^7	6.1x10^{-1}	7.6	Iacoboni and LeBrun (May 1978)
150-cm depth				4.5x10^2	4.7	
Middle 120-cm depth	36-58°	9	2.9x10^6	1.2x10^1	5.4	Iacoboni (November 1977)
30-cm depth	43-59°	9	1.7x10^7	1.0x10^2	5.2	Iacoboni and LeBrun (June 1978)
150-cm depth				1.0x10^2	5.2	
Bottom				1.0x10^7	0.2	

* NS = Not specified by author

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bacteria to 10^6 to 10^8 MPN/GDW in compost, which was then exposed to temperatures of up to 58°C for 32 minutes; reductions in density were insignificant, at less than one order of magnitude. Under similar test conditions, coliform bacteria were reduced by 1 log in 8 minutes at 52°C.

Bacillus cereus can be considered an indicator of the genera of bacteria which includes the causative agent of anthrax. Krogstad and Gudding (1975) inoculated a 1:1 mixture of refuse and sludge with *B. cereus* at densities of 10^5 to 10^6 MPN/GDW, then fed the compost mass into a pilot-scale mechanical composting unit, or reactor. After five runs of composting of 7 days duration each, *B. cereus* was present in all samples taken at periodic intervals during the composting process, except those from one run in which temperatures had exceeded 70°C during the first 36 to 48 hr.

Pathogenic Bacteria. Most work on survival of pathogenic bacteria in composting sludge has focused on *Salmonella* spp. Researchers have, however, also looked at *Serratia marcescens* (Krogstad and Gudding, 1975), *Staphylococcus aureus* and *Shigella sonnei* (Gaby, 1975), and *Candida albicans* (Wiley and Westerberg, 1969).

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Data obtained in laboratory-scale or pilot-type tests on *Salmonella* densities are presented in Figure 15 (Ward and Brandon, 1977). The data pertain primarily to thermal effects of composting on these bacteria. Burge *et al.* (1978) determined that *S. typhimurium* held at 55°C had a "D" value (the time required to effect a 1-log reduction) of 80 minutes. Their findings for this species are consistent with those of Krogstad and Gudding (1975), who composted in a pilot reactor a 1:1 mixture of sludge:refuse that had been inoculated with densities of *S. typhimurium* of 10^5 to 10^6 per gram dry weight. The bacteria were found to be present in most or all samples until temperatures of 60 to 65°C were attained after 72 hours, at which point *S. typhimurium* could be detected in no samples.

Other *Salmonella* species may be less resistant to the process. *S. enteritidis* populations inoculated into composted sludge at densities of 10^6 to 10^8 MPN/GDW have been inactivated by 1 order of magnitude within 45 minutes of exposure to a temperature of 45°C and by 4 to 6 orders of magnitude within 30 to 40 minutes at 52°C or within 10 minutes or more at 54°C (Ward and Brandon, 1977). Wiley and Westerberg (1969) reported total inactivation of *S. newport* after 40 minutes at 60°C.

Gaby (1975) inserted into open refuse/sludge windrows sealed culture vials containing densities on the order of 10^7 MPN/GDW of *S. typhimurium*. He recorded possible proliferation of this species over a range of 25° to 45°C, over a period of as much as 14 days. A 6-log reduction in density was observed, however, when mid-depth temperatures reached highs of 50°C over 28 days in winter conditions and of 55° to 60°C over 4 days in summer conditions.

Results obtained at full-scale composting operations are present in Tables 47 and 48. At forced-aeration installations studied by Epstein *et al.* (1976) and Iacoboni and LeBrun (1978), log reductions in *Salmonella* were seen to

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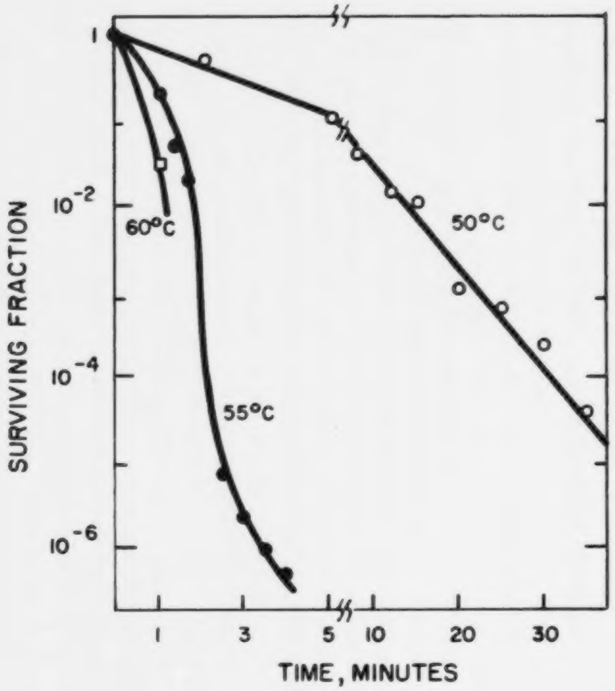

FIGURE 15. HEAT INACTIVATION OF SALMONELLAS IN LIQUID DIGESTED SLUDGE *

* WARD AND BRANDON (1977)

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TABLE 47. DENSITIES OF SALMONELLA IN FORCED AERATION COMPOSTING SYSTEMS

Sampling Point	Temperature (°C)	Duration (days)	Initial Density (MPN/gdw)	Final Density (MPN/gdw)	Log Reduction	Reference
Toe	15-46°	28	37	<6	>0.08	Epstein *et al*. (1976)
Top, middle, bottom	26-48°	25	1.95×10^4	≤10	>3.3	Iacoboni and LeBrun (1978)
Toe	42-58°	28	<10	<10	-	Epstein *et al*. (1976)
40-cm center	15-60	28	37	<8	>0.7	Epstein *et al*. (1976)
40-cm center	28-60	28	53	0.3	2.2	Epstein *et al*. (1976)
Toe	48-70	28	53	1.1	1.7	Epstein *et al*. (1976)

TABLE 48. DENSITIES OF SALMONELLA IN DEEP-PILE BIN COMPOSTING SYSTEMS

Temperature (°C)	Duration (days)	Initial Density (MPN/gdw)	Final Density (MPN/gdw)	Log Reduction	Reference
36-43°	30 (17 composting, 14 storage)	1.3×10^4	<0.1	>5.1	Iacoboni and Livingston (1977)
34° ave. 58° max.	24	9.3×10^4	<0.2 (30-cm) <0.21 (150-cm)	5.7	Iacoboni and LeBrun (May 1978)
36-58°	9	1.4×10^5	4.9×10^3	1.5	Iacoboni (November 1977)
43-59°	9	4.8×10^4	<0.4-38	3.1-5.1	Iacoboni and LeBrun (June 1978)

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range from less than 1 to more than 3 (Table 47). In all cases, the tests involved composting at temperatures in the mesophilic range over periods of 25 to 28 days. Final densities of *Salmonella* in all cases were at levels of 10 MPN/GDW or less; variation in log reductions can be explained by variation in the low initial densities and by the sample location. Samples from the toe of the static pile system where low temperatures occur, would be expected to produce higher counts.

Iacoboni (November 1977) reported a high final density (4.9×10^3 MPN/GDW) of *Salmonella* after 9 days of composting sludge in deep-pile bins. This does not agree, however, with results of three other tests carried out at the same facility under quite similar conditions (Table 48), in which reductions of 3.1 to 5.7 logs resulted in final *Salmonella* counts of only 0.1 to 38 per gram dry weight.

Studies of densities of other pathogens in composting sludge can be summarized as follows:

a. Gaby (1975) inserted into refuse:sludge open windrows sealed culture vials containing populations of 10^6 to 10^7 *Staphylococcus aureus* per gram dry weight. Results (Table 49) followed closely those obtained for fecal coliform bacteria in the same series of tests: the populations thrived for 1 to 14 days at temperatures of less than 45°C but dropped by 3 to 5 orders of magnitude once temperatures above 50°C were reached. (The controlled conditions of Gaby's insertion experiments may make them useful as indicators of thermal sensitivity, but not as accurate tests of effects of composting. Whatever non-thermal effects may contribute to bacterial kill--drying, reduction of food source, generation of antibiotic substances in the pile--were effectively excluded by the sealed vials. The increases in all bacterial populations observed by Gaby at moderate temperatures probably resulted from the placement of bacteria in a nutrient broth.)

b. In the same experiment, Gaby (1975) obtained similar results for *Shigella sonnei*. Results for the organisms tested are presented in Table 49.

c. Populations of *Serratia marcescens* were inoculated by Krogstad and Gudding (1975) into a 1:1 sludge: refuse mixture at densities of 10^5 to 10^6 per gram dry weight. Fed into a pilot-scale mechanical composting unit, the compost mass contained *S. marcescens* after 24 hr at 45°C, but none after several hours at higher temperatures.

d. Wiley and Westerberg (1969), in a laboratory demonstration of mechanical composting, found *Candida albicans* to be totally inactivated after 60 min at temperatures of 70°C.

Viruses. Most studies of virus inactivation by composting have involved seeding viruses into sludge prior to mixing and forming the compost piles. For example, Cramer and Burge (1975) seeded digested sludge with the indicator virus bacteriophage f2 prior to dewatering the sludge and mixing it with

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TABLE 49. REDUCTIONS IN DENSITIES OF TWO PATHOGENIC SPECIES INSERTED INTO SURFACE AND MID-DEPTH OF OPEN-WINDROW PILES*

Pathogen Species	Composting Season	Temperature (°C)	Days After Start	Initial Density (MPN/gdw)	Final Density (MPN/gdw)	Log Reduction
Shigella sonnei	Winter	25°	7	10^7	10^7-10^8	0 to -1**
		35-45°	14		10^7	-
		50-60°	28		10^1	6
	Summer	41-45°	1	10^7	10^1-10^8	-1 to 6**
		55-60°	4		10^1	6
Staphylococcus aureus	Winter	25°	7	10^6	10^6-10^7	0 to -1**
		35-45°	14		10^7-10^8	-1 to -2**
		50-60°	28		10^1-10^3	3 to 5
	Summer	41-45°	1	10^6	10^5-10^8	
		55-60°	4		10^1	5

*From data of Gaby (1975). Sealed culture vials containing bacteria were inserted into windrows; initial increases in density would be expected due to nutrient medium.

**Densities varied with depth of sample, but no consistent relationship is evident between depth of sample and density, nor between temperature at sampling point and density.

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woodchips for forced-aeration composting. Measuring at three depths within the static piles, the researchers found log reductions of 0.9 to 2.7 over a range of seasonal operating conditions that included composting for 28 days with a mean high pile temperature of 22°C in winter, to composting for 14 days with a mean high temperature of 68°C in summer. Log survivals for composting are presented in Table 50. Time/temperature relationships are evident: composting for 14 days with a high temperature of 68°C achieved as good a reduction in viral titer as composting for 28 days with a high temperature of 22°C.

Time may, in fact, be the more important factor in determining bacteriophage f2 survival. Storage of the compost for 42 days after composting reduced bacteriophage f2 counts by 7 logs from their original levels, even though temperatures reportedly never exceeded 22°C.

The effects of heat, in composting, may be highly variable according to the type of virus being considered. Burge *et al*. (1978), in laboratory-based assessment of "D-values" for viruses, determined that 267 min at 55°C would yield a 1-log reduction in f2 bacteriophage. The same team reported an 11-min D value for adenovirus 12 NIAID (a pathogenic virus), and a 1.8-min D value for poliovirus type 1. Wiley and Westerberg (1969) reported that temperatures of 60°C effectively inactivated poliovirus type 1 after 5 min exposure.

Ward and Ashley (1978) heated sludge samples of varying solids' concentrations and found a clear, direct relationship between solids and virus survival. Poliovirus type 1 strain CHAT seeded into the samples was reduced by 2.2 logs when heated at 51°C for 2 min in sludge of 5-percent solids, by 2.3 logs at the same temperature for 5 min in sludge of 40-percent solids, and by only 1.5 logs after 100 min in sludge of 80-percent solids. Typical results from the mesophilic range are presented in Tables 51 and 52.

Ward and Ashley also compared inactivation of poliovirus type 1 in raw and composted sludge, heating the samples to 39°C and 43°C. Inactivation was found to be much more rapid in the composted sludge, as shown in Table 53. Precisely the opposite effect was witnessed in a similar test by this team on reovirus type 3 strain Dearing, at 45°C: reovirus was inactivated much more quickly in raw sludge than in composted sludge.

Parasites. Wiley and Westerberg (1969) have shown total inactivation of *Ascaris lumbricoides* ova after 60-min exposure to 60°C temperature in laboratory-scale mechanical composting. Such conditions would not necessarily occur within the minimum time-temperature boundaries of "mesophilic composting". In exposed windrows operated at temperatures only slightly lower, some ova have been observed to remain viable.

Iacaboni and LeBrun (May 1978) obtained 2.04 viable ova per GDW at 30 cm depth and less than 1.63 per GDW at 150 cm in a deep-pile bin composter operated for 24 days at an average temperature of 34°C (58°C single highest recorded temperature). No initial concentrations were reported in this study. Horvath (1977) recovered some viable ova after 69 days in 120 cm

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TABLE 50. DENSITIES OF BACTERIOPHAGE F2 IN FORCED-AERATION WINDROWS AT BELTSVILLE, MARYLAND*

Season	Mean High Pile Temperature (°C)	Time (Days)	Initial Log Density	Final Log Density	Log Reduction
Spring	41	21	7.5	5.2-6.5	1.0-2.3
Summer	49	16	7.8	5.2-6.9	0.9-2.6
Fall	68	14	7.6	4.9-6.0	1.6-2.7
Winter	22	28	7.0	4.9-6.0	1.0-2.1

*Cramer and Burge (1975). Solids concentrations not given.

TABLE 51. REDUCTIONS OF POLIOVIRUS TYPE 1 STRAIN CHAT IN RAW SLUDGE HEATED AT 47°C*

Solids' Concentration (%)	Time (min)	Log Reduction
5	20	2.3
	40	2.8
	60	2.9
40	50	0.8
	100	1.0
	150	1.3
	200	1.5
80	100	0.3
	200	0.6

*Ward and Ashley (1978)

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TABLE 52. REDUCTIONS OF POLIOVIRUS TYPE 1 STRAIN CHAT IN RAW SLUDGE HEATED AT 51°C

Solids' Concentration (%)	Time (min)	Log Reduction
5	2	2.2
	5	5.2
40	5	2.3
	20	4.5
80	20	0.8
	50	1.2
	100	1.5

*Ward and Ashley (1978)

TABLE 53. INACTIVATION OF POLIOVIRUS TYPE 1 STRAIN CHAT IN RAW AND COMPOSTED SLUDGE AT 40% SOLIDS*

Sludge Type	Temperature (°C)	Time (min)	Log Reduction
Dewatered	39	100	0.2
		200	0.3
	43	100	0.3
		200	0.5
Composted	39	20	1.0
		50	1.6
		100	1.8
		200	2.1
	43	5	2.5
		10	3.2
		20	3.5
		50	3.8
		100	4.6

*From data of Ward and Ashley (1978)

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deep windrows, internal temperatures of which reached highs of 54° to 58°C; the windrows were turned daily. Krige (1964) recorded mixed results from a number of refuse/sludge composting operations in South Africa: no ova were recovered from an open compost system where material was held more than 200 days at temperatures of up to 50°C, but 21 to 50 ova (half of them viable) per gram were recovered from an operation in which composting was continued for 56 days at up to 53°C, and intermediate counts (1 to 20 ova per gram) were recovered at yet another operation involving 60 days' composting at up to 50°C.

Brannen *et al.* (1975) reported no significant reduction of *A. lumbricoides* ova suspended in sludge supernatant (1% solids) after more than 2 hr at 47°C, but noted a 1-log reduction after 13 to 15 min at 51°C and after 0.5 min at 55°C. Similarily, Brandon (1978) observed little effect on embryonation of *Ascaris* ova at 45°C in a compost system, but he found a 3-log reduction in embryonation within 50 min at 55°C.

Cysts of *Entamoeba histolytica* are reported by Cramer and Burge (1975) to be quite resistant to temperatures in the mesophilic range, but Burge *et al.* (1978) cites Chang (1950) in reporting a D-value for these cysts of only 44 min at 55°C.

Other Pathogenic Microorganisms. The fungus *Aspergillus fumigatus* grows on hot (50° to 60°C) working compost piles as well as in natural environments. Concern has been expressed that operations at a sludge composting site might tend to disperse this fungus in air currents, tending to infect susceptible persons. Millner *et al.* (1977) took samples from three temperature zones in forced-aeration static piles before and after composting. Before composting, samples were made not only of the vacuum-filtered sludge, but also of new woodchips and previously-composted woodchips that were to be mixed with the sludge as a bulking agent. Levels of *A. fumigatus* in sludge were 10^2 to 10^3 CFU/GDW; in fresh woodchips, 10^7 to 2.3×10^5 CFU/GDW; and in recycled woodchips, 2.6×10^6 to 6.1×10^7 CFU/GDW.

After composting for 21 days, the sludge was sampled from three temperature zones. Results are presented in Table 54.

It is not possible to show log reductions, since initial densities in the sludge/woodchip mixture are not reported. Subsequent sampling by the Millner team of the curing and storage piles indicated that *A. fumigatus* from lower-temperature zones may have reinoculated the fungus-free compost from high-temperature zones; levels of *A. fumigatus* were easily detectable at levels of 10^3 to 10^5 CFU/GDW until the sixth month of storage, when only negligible levels--if any--could be detected in samples.

Discussion. The temperatures of mesophilic composting are immediately lethal to few, if any, of the microorganisms of concern in sludge. In fact, the process' moderately high temperatures may actually support growth of certain bacterial populations (Cooper and Golueke, 1978) and the fungus *Aspergillus fumigatus* (Millner *et al.*, 1977). The mixing of compost that occurs when it is moved for curing and storage can redistribute the viable bacteria and

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TABLE 54. DENSITIES OF ASPERGILLUS FUMIGATUS IN THREE TEMPERATURE ZONES OF STATIC-PILE WINDROWS*

Temperature (°C)	No. of Samples	Solids' Concentration	Density (CFU/GDW)
< 40	2	49 - 51%	2.9×10^3 to 4.5×10^4
40 - 60	6	51 - 69%	$<10^3$ to $> 5.0\times10^5$
> 60	1	72%	0

*Millner *et al*.(1977)

fungi into zones where significant reductions occurred, and reactivation of populations of indicator and pathogenic bacteria and *A. fumigatus* can take place (Horvath, 1977; Brandon *et al*., 1977; Millner *et al*., 1977). On the basis of studies in which viruses were seeded into composting piles, pathogenic viruses, although exceedingly difficult to monitor, are relatively easy to control; most are quite susceptible to the conditions of mesophilic composting, if the process is properly operated, and, because they multiply only in a host cell system, will not "regrow" once inactivated.

A summary of the effects of mesophilic composting or indicator and pathogenic organisms follows:

Indicator Bacteria. The indicator bacteria that have been most studied are total coliform, fecal coliform, and fecal streptococcus bacteria. Total coliform bacteria decline in numbers by more than 3 logs in composting systems meeting the requirements for "process to significantly reduce pathogens." Caution must be taken, however, to ensure that all the sludge being composted reaches the required temperature and time conditions. Studies show that some areas of composting systems, such as surfaces and edges, do not always meet the requirements.

All the test results shown in Tables 45 and 46, except for one set of results, show decreases of 4 logs or more in numbers of fecal coliform bacteria at composting temperatures from 15 to 70°C. The one exception is for a sample collected from the bottom of a deep-pile bin composting system (Iacoboni and LeBrun, 1978). Temperatures at that location might not have reached the required levels. The data do not indicate, however, to what degree composting installations that marginally conform to the definition for "processes to significantly reduce pathogens" would actually decrease fecal coliforms.

Fecal streptococcus bacteria appear to be quite resistant to conditions of mesophilic composting. Laboratory studies by Ward and Brandon (1977) showed no significant reduction in densities of these bacteria at up to 32 min exposure at 58°C. Full-scale composting studies by Iacoboni (November 1977) yielded reductions of 6.5 log after 9 days composting at the mesophilic/thermophilic boundary. It should be noted, however, that Iacoboni's final samples (after 26 days composting) contained densities of 4.0×10^3 fecal streptococcus bacteria, yielding only a 2.9-log reduction overall and indicating significant regrowth after the initial kills.

Pathogenic Bacteria. *Salmonella* populations are reduced to negligible densities (less than or equal to 10 MPN/GDW) under the temperature conditions of mesophilic composting as defined in 40 CFR 257. This can be seen in laboratory data in which exposure to 55°C temperatures for 80 min yields a 1-log reduction in *Salmonella enteritidis* serotype Senftenberg 77W (Burge *et al*., 1978) and in which heating of inoculated compost samples to 50°C results in 1.1-log reduction of *S. enteritidis* serotype Montevideo within 40 min (Ward and Brandon, 1977). Less than 1-hr exposure of *S. typhi* to temperatures of 55° to 60°C will result in 3-log reduction of density (Cramer and Burge, 1975).

The implication from laboratory data is that the required exposure to temperatures of at least 55°C for at least 4 hr during the compost cycle will inactivate significant numbers of Salmonella. This implication appears to be borne out in full-scale experience at composting plants, where final densities of Salmonella are exceedingly low (Tables 47 and 48). Unfortunately, in all but two of the full-scale experiments reviewed for the report, densities were recorded over composting periods of more than 3 weeks. In only two instances (Iacoboni, November 1977, and Iacoboni and LeBrun, June 1978) were shorter composting periods--9 days in each case--tested. Results in these tests of shorter duration resulted in greater than 1 log reductions, with final densities of 4900 MPN/GDW at 36° to 58°C (Iacoboni) and <0.4 to 38 MPN/GDW at 43° to 59°C (Iacoboni and LeBrun) were recorded.

Among other pathogenic bacteria:

- Mycobacterium tuberculosis var hominis will almost certainly survive mesophilic composting (Morgan and MacDonald, 1969) but at what density is unknown. A temperature of 65° to 66°C will reduce the species' density by 3 logs in minutes (Cramer and Burge, 1975).
- Serratia marcescens appears to be inactivated quickly at temperatures greater than 45°C (Krogstad and Gudding, 1975) and so is susceptible to mesophilic composting.
- Shigella sonnei and Staphylococcus aureus have been reduced by 6 and 5 logs, respectively, under conditions that closely approximated the temperature/time conditions of mesophilic composting (Gaby, 1975).

Viruses. Most viruses of concern in sludge appear to be quite vulnerable to temperature conditions of composting. The echovirus, reovirus and coxsackie virus genera can be reduced by three orders of magnitude by temperatures within the mesophilic range, as can the adenovirudae (Cramer and Burge, 1975: see Figure 16). Poliovirus shows similar susceptibility in sludge of 40-percent solids concentration: Ward and Ashley (1978) noted a 4.5-log reduction in poliovirus type 1 strain CHAT exposed to 51°C temperatures for 20 min. Cooper and Golueke (1978) reported a 1.8-log reduction in poliovirus type 1 strain LSc within 48 hr of startup in a small-scale compost system (temperature not known). In fact, of the viruses studied in research reviewed for this report, only bacteriophage f2 (a non-pathogenic virus) showed marked resistance to mesophilic temperatures. Burge et al. (1978), who cited the species' hardiness as one measure of its value as a viral indicator, reported a 267-min detention time was necessary to achieve a 1-log reduction in its density at 55°C. One can infer that a 1-log reduction in bacteriophage f2 would indicate effective inactivation of enteroviruses and other pathogenic viruses.

The only quantitive data from full-scale composting facilities neither fully support nor refute the laboratory evidence on effectiveness of mesophilic composting. Cramer and Burge (1975) seeded bacteriophage f2 into forced-aeration windrows and consistently obtained reductions of 1 to 2 logs in densities through a range of mean high temperatures of 22° to 68°, over a range

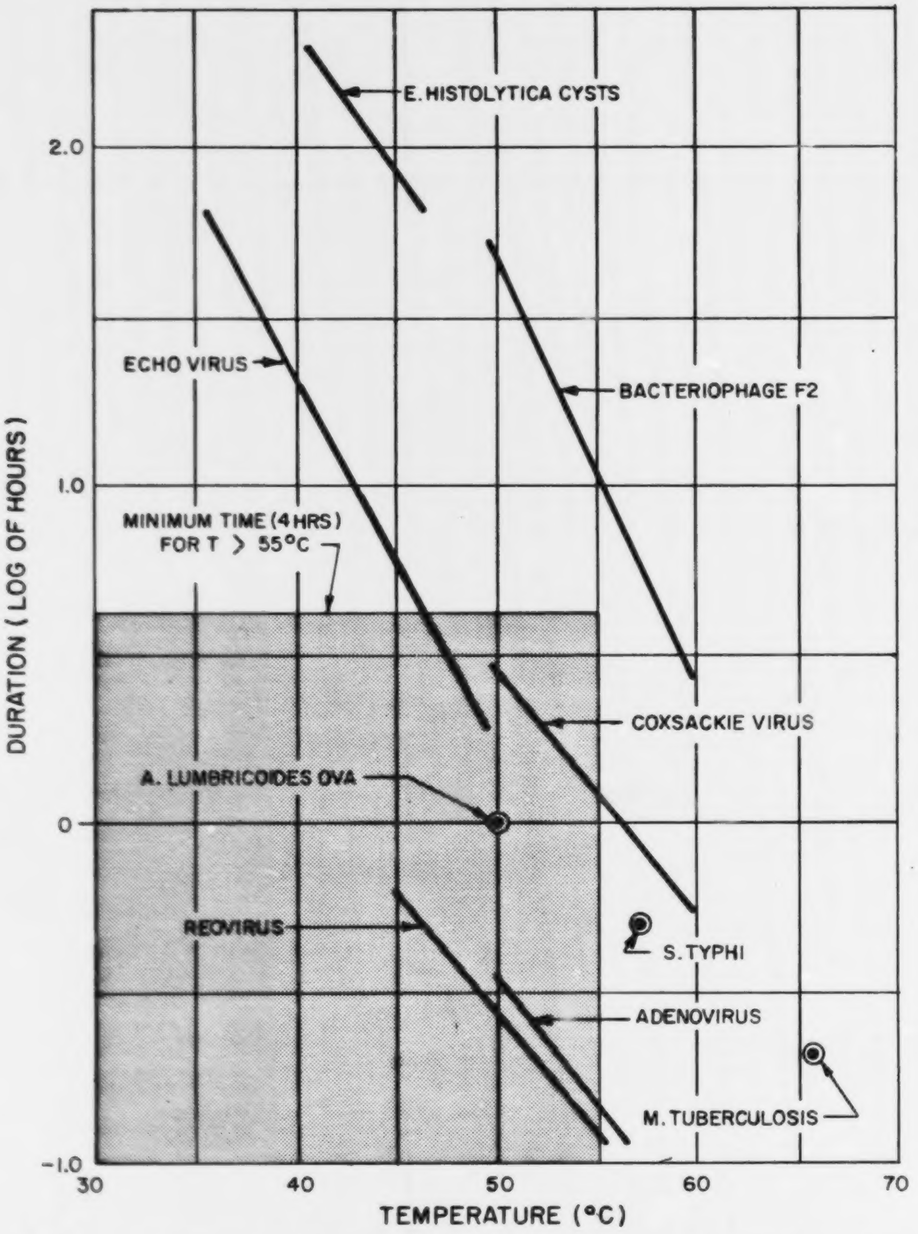

FIGURE 16. TEMPERATURE/TIME RELATIONSHIPS FOR 99.9 % REDUCTIONS OF INDICATORS, PATHOGENS AND PARASITES.*

*CRAMER AND BURGE (1975)

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of composting times of 14 to 28 days. The data appear to indicate that time is as important as temperature. As the durations studied by Cramer and Burge are 3 to 5 times longer than those set as minimum for mesophilic composting, there is no certainty that significant reductions of indicator and pathogenic viruses will occur in the "mesophilic" systems.

Moreover, Ward and Ashley (1978) have drawn some interesting conclusions about factors other than temperature that can influence virus survival. (Their report, unfortunately, contains no details on sample size, number of tests, or actual results.) Measuring heat inactivation of poliovirus type 1 strain CHAT in raw sludge samples of 5, 40 and 80-percent solids concentrations, Ward and Ashley found inactivation to be inversely related to solids concentration (Figure 17). Nevertheless, poliovirus in sludge of 40-percent solids--approaching the concentration that would be expected in fresh makeup for composting rows--was quickly inactivated at 51°C.

Ward and Ashley also compared survival of poliovirus type 1 strain CHAT and reovirus type 3 strain Dearing in raw and composted sludge. Markedly different results were obtained. Poliovirus in composted sludge was inactivated by 2 to 3 orders of magnitude more than poliovirus in raw sludge at 39° and 43°C (Figure 18). The reverse was true for the reovirus; inactivation was 3 logs greater in the raw sludge (Figure 19). The authors have hypothesized that a detergent present in raw sludge may protect poliovirus from heat inactivation while increasing reovirus' susceptibility to heat inactivation. The detergent, they suggest, is destroyed in the composting process, and its effects, therefore, are lost. It should be noted that as the researchers used samples of composted sludge from the Beltsville forced-aeration system, there can be no assurance that similar results would have been obtained within a mesophilic composting system, where maximum temperatures are considerably lower and duration of composting can be as short as 5 days.

Parasites. Cramer and Burge (1975) report a 3-log reduction in densities of *Ascaris lumbricoides* ova at 1 hr and 50°C. Laboratory-scale work by Brannen *et al. (1975)* supports their data, showing a 1-log reduction in viable ova in a sludge-supernate (1% solids) held at 51°C for 15 minutes. This would imply the near-total inactivation of these ova in mesophilic composting, since initial densities of these ova in sludge are rarely more than one order of magnitude. Several other authors, however, have reported data from full-scale installations that show survival of *A. lumbricoides* ova over a range of harsher conditions. Horvath (1977), for example, saw survival of viable ova even after 69 days of composting in 120 cm deep windrows at temperatures of as high as 58°C. Krige (1969) reported survival of ova in two of three open compost sites in South Africa where pile temperatures had been maintained at 50° to 53°C; each of the two had been composting for 50 to 60 days, but at the third, where no ova were detected, composting had continued for over 200 days.

Other Pathogenic Microorganisms. Mesophilic composting will not significantly reduce densities of the fungus *Aspergillus fumigatus*. In fact, the temperatures encountered in this process are optimum for the organism's growth. Control of health risks asociated with *A. fumigatus* must come from

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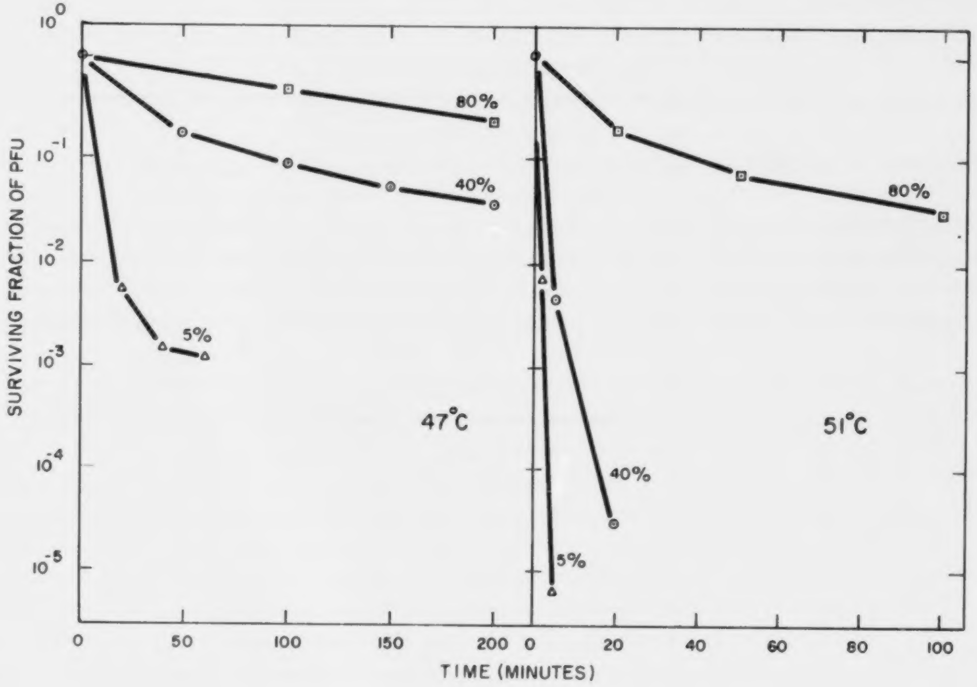

FIGURE 17. HEAT INACTIVATION OF POLIOVIRUS TYPE I STRAIN CHAT IN RAW SLUDGE SAMPLES OF 5, 40 AND 80% SOLIDS CONCENTRATIONS AT 47° AND 51°.*

* FROM WARD AND ASHLEY (1978)

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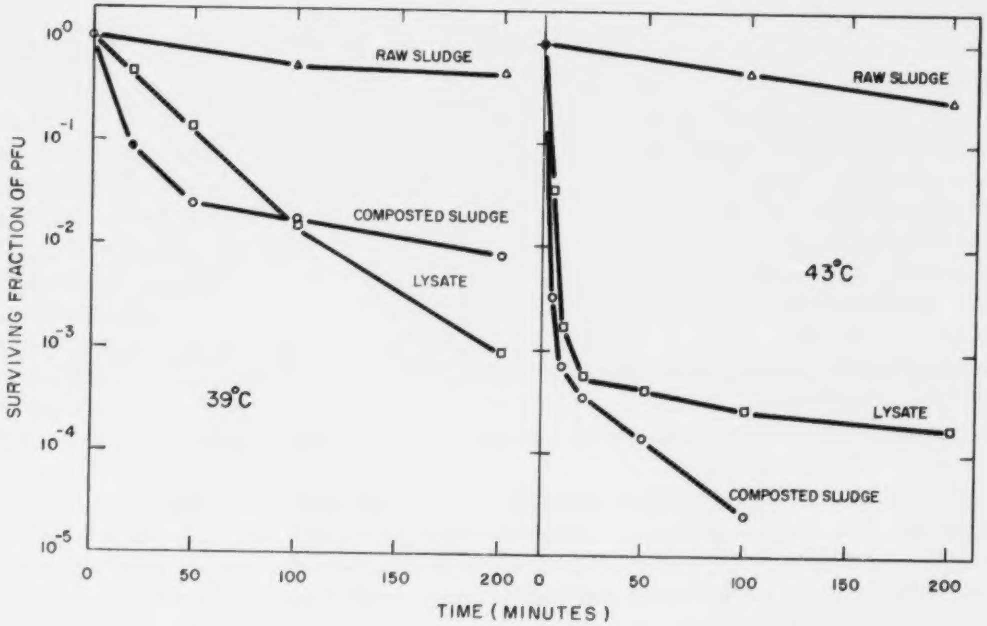

FIGURE 18. HEAT INACTIVATION OF POLIOVIRUS TYPE I STRAIN CHAT IN RAW SLUDGE, COMPOSTED SLUDGE AND LYSATE AT 39°C AND 43°C.*

* FROM WARD AND ASHLEY (1978)

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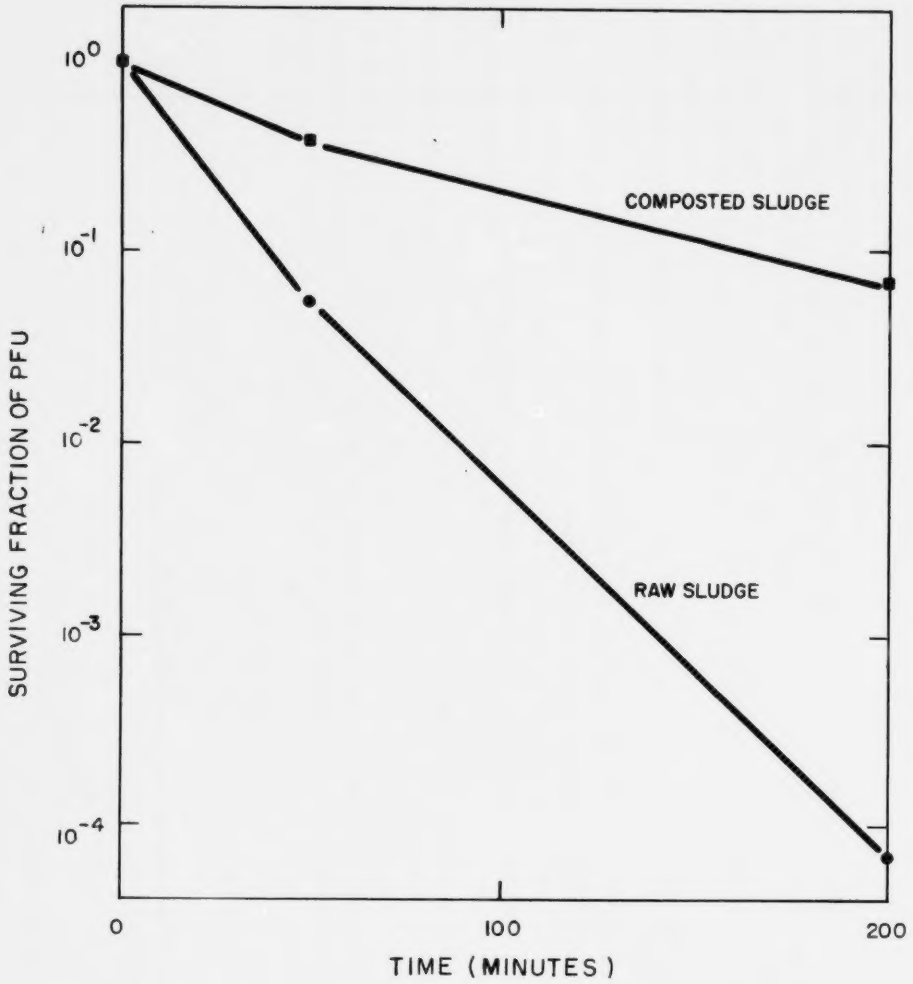

FIGURE 19. HEAT INACTIVATION OF REOVIRUS TYPE 3 STRAIN DEARING IN RAW AND COMPOSTED SLUDGE AT 45° C.*

* FROM WARD AND ASHLEY (1978)

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appropriate choice of siting and from operational steps--such as control of dust levels, and measures to control inoculation of fresh sludge--designed to reduce exposure of workers and the general public.

Factors Influencing Reductions on Microorganism Densities. Most investigators have studied reductions of microorganisms in composting from the standpoint of heat inactivation as the limiting factor. The success of the system in reducing pathogens through heat inactivation, however, depends entirely on achieving the inactivation temperature throughout the pile.

In the closed composting system, it is relatively easy to assure that desired temperatures are reached uniformly throughout the compost mass, provided that the moisture content, carbon-to-nitrogen (C:N) ratio, and initial pH all lie within satisfactory ranges. This type of system is usually well protected from ambient conditions.

In open-windrow or forced-aeration systems, maintenance of uniformly high temperatures is problematic. Cold weather and heavy rainfalls can lower temperatures within the compost pile, both directly--and indirectly, by slowing the feeding activity of decomposer microbes. Lower temperatures tend to result in poorer pathogen kills and reactivation of bacterial and fungal populations.

The "toe" or lower outer edge of a static pile used in forced-aeration composting typically remains cooler, due to its high area:volume ratio, than the inner portion of the pile (Epstein, 1976). Examination of data in Table 43 shows a much higher log reduction of total coliform bacteria from internal portions of a pile than from either the "toe" or top of the pile.

In open-windrow composting, a repeated cycle of sudden heating and gradual cooling is seen when windrows are turned and aeration thereby temporarily improved. Nevertheless, at any given time, it is quite possible for temperatures to be within lethal ranges in only 30 percent of the pile mass, with temperatures at levels optimum or, at least, satisfactory for many organisms throughout the rest of the pile (Cooper and Golueke, 1978).

Aside from pile temperature, factors that may contribute to reduction of microorganism populations in composting are production of antibiotic substances (Krogstad and Gudding, 1975), presence of antiviral substances such as ammonia in the pile (Ward and Ashley, 1978), and reduced nutrient availability (Cooper and Golueke, 1978). No conclusive results exist, however, to link these factors to the performance of the mesophilic composting process.

LIME STABILIZATION

Introduction

Description of Process. As defined by EPA's proposed Criteria (40 CFR Part 257), lime stabilization involves applying lime to sludge in quantities sufficient to raise sludge pH to 12.0 for at least 2 hours.

The technique differs from digestion or composting of sludge in that it does not affect bacterial food sources in the sludge but instead simply kills off many microorganisms due to the high pH levels attained. Several researchers have reported substantial initial kills of indicator and pathogenic bacteria in sludges treated by lime stabilization. The initial pH level attained drops significantly over the next few hours, however, and some bacterial populations are able to regrow in the sludge mass.

At full-scale installations, wastewater-treatment plant operators practice at least three methods of lime stabilization (Camp Dresser & McKee, 1980):

a. Addition of lime prior to dewatering--Many treatment plants have provision for conditioning of sludge by addition of lime prior to dewatering. Some operators have chosen simply to increase the lime dose to effect lime stabilization. If carried out properly, this practice will give about 15 minutes detention time for the mixture of lime and liquid sludge; the sludge will then be dewatered, and the cake will have a pH of 12.0 for the remainder of the 2-hr detention period.

b. Batch operation--Lime can be added directly to a mixed-sludge storage tank. The sludge is not dewatered but instead is applied directly to land.

c. Addition of lime to sludge cake--This technique has been observed in practice, but no studies concerning its effectiveness have been found in the literature.

In the lime-stabilization investigations reported in other literature, lime is added to a liquid sludge. The sludge is held in a liquid form for the detention period of the test. This pilot- or laboratory-scale technique is most analogous to the second (b.) method of the three used at full-scale installations.

Operating Parameters. Parameters that can be varied in lime stabilization are pH level and detention time. High pH is the killing agent, and variation in detention time will govern the degree of sludge-solids contact in a high pH environment.

The lime dose necessary to effect a specified pH has been found to vary as a function of sludge-solids concentration (Counts and Shuckrow, 1974) and sludge type (Noland *et al.*, 1978). Lime doses used in the latter study to achieve a pH of 12.0 ranged from 0.12 to 0.30 parts CaO per part (by weight) of dry sludge solids.

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Data Base Available. Most of the data amenable to quantitative analysis came from a very limited number of reasonably extensive laboratory- and pilot-scale studies, notably those of Counts and Shuckrow (1974), Farrell *et al*. (1974), Feige *et al*. (1975), Noland *et al*. (1978), and Reimers *et al*. (1980). Following are brief descriptions of key studies:

a. Laboratory-scale tests--These tests involved titration of sludge samples with lime solution.

- Counts and Shuckrow (1974)--pH (11.0-12.4), detention time (1 hr and 24 hr), and sludge-solids concentration (2.0 and 4.4 percent solids) were studied with respect to effects on inactivation of fecal coliform bacteria, fecal streptococcus bacteria, *Salmonella* spp., and *Pseudomonas aeruginosa* by lime stabilization. During the detention period, samples were stored in open beakers at room temperature.

- Farrell *et al*. (1974)--Three pH levels (10.5, 11.5, 12.5) and two detention times (0.5 and 24.0 hr) were studied with respect to effects on fecal coliform bacteria, fecal streptoccus bacteria, *Salmonella* spp., and *Pseudomonas aeruginosa* in a chemically precipitated primary sludge at 5-percent solids concentration.

- Reimers *et al*. (1980)--These studies were conducted on primary sludge and on sludges from two laboratory aerobic digesters. One of the aerobic digesters operated at 28°C; the other at 35°C. Both had 10-day hydraulic detention times. Lime dosages as CaO were equal to 8, 76, and 230 percent of the suspended-solids concentrations. These dosages brought the pH to between 8.7 and 13.4. Some samples were aerated continuously after lime addition. Others were kept under anaerobic conditions.

  Analyses for viable *Ascaris* eggs were conducted from 1 hr to 20 days after lime addition.

  This study also showed distinct differences in the resistance shown by *Ascaris* ova isolated from different sources. *Ascaris* eggs derived from the uteri of worms are easier to inactivate as compared to *Ascaris* eggs taken from the intestinal content of swine. (Personal Communication)

- Sattar *et al*. (1976)--One-liter aliquots of poliovirus-seeded sewage were raised to pH 11.5, stirred for 0.5 hr, and allowed to sit for 1 hr; the samples were then tested for presence of poliovirus.

b. Pilot-scale tests--In these tests, lime was added to a mixed sludge storage tank operating in a batch mode.

- Counts and Shuckrow (1974)--Sufficient lime was added to three types of sludge--primary sludge, trickling-filter humus, and mixed primary-sludge/trickling-filter-humus--to achieve a pH of 12.0 after 0.5 hr. Samples were tested for fecal coliform bacteria, fecal streptococcus bacteria, *Salmonella* spp., and *Pseudomonas aeruginosa*.

- Feige *et al*. (1975)--Lime slurry was added to septage in a 4,500-gal holding tank in doses sufficient to bring pH to 11.5 prior to discharge to sand drying beds. Samples were tested for fecal coliform and fecal streptococcus bacteria.

Only two of the above studies (Farrell *et al*. and Reimers *et al*.) evaluated the effects, if any, of dewatering limed sludge within the two-hour detention period--a practice that is relatively common at full-scale installations. This portion of the Farrell *et al*. study is addressed in Section 5, under lime conditioning for mechanical dewatering.

Results

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Indicator Bacteria. Densities of indicator bacteria in lime-stabilized sludge have been studied with respect to variables of sludge type, pH, detention time, and sludge-solids concentration.

a. Sludge type--Counts and Shuckrow (1974) examined survival of fecal coliform and fecal streptococcus bacteria following pilot-scale lime stabilization of three sludge types--primary sludge, trickling-filter humus, and mixed primary sludge and trickling filter humus. Samples were raised to a pH of 12.0 or greater for 0.5 hr detention time; results are presented in Table 55. Both types of indicator bacteria are seen to be reduced by 3- to 6-log orders under the test conditions.

   In a similar study, Noland *et al*. (1978) observed 1- to 6-log reductions in densities of total coliform bacteria, fecal coliform bacteria, and fecal streptococcus bacteria in four types of sludge limed to pH of 12.0 for total detention times (mixing plus holding) of 1.4 to 4.1 hr. Results are presented in Table 56.

   Feige *et al*. (1975) studied survival of fecal coliform and fecal streptococcus bacteria in septage treated by lime stabilization to pH 11.5 for a detention time only as long as required to load the lime slurry. Results are presented in Table 57. Fecal coliform bacteria were reduced by three orders of magnitude; fecal streptococcus bacteria by one order of magnitude. Although reductions observed by Feige *et al*. are more moderate than those recorded by Counts and Shuckrow or Noland *et al*., final densities of fecal coliform bacteria are on the same order in all three studies. Better removals of fecal streptococcus bacteria were observed in the higher-pH conditions of tests conducted by Counts and Shuckrow and Noland *et al*., however.

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TABLE 55. DENSITIES OF INDICATOR BACTERIA IN THREE TYPES OF SLUDGE BEFORE AND AFTER LIME-STABILIZATION TREATMENT*
(Numbers/100 ml)

Sludge Type	Fecal Coliform Bacteria		Fecal Streptococcus Bacteria	
	Range	Mean	Range	Mean
Primary				
Raw	$8.4 \times 10^5 - 2.1 \times 10^7$	6.8×10^6	$2.2 \times 10^6 - 4.9 \times 10^6$	3.7×10^6
Treated	$2.9 \times 10^2 - 6.4 \times 10^3$	7.0×10^2	$1.0 \times 10^0 - 3.0 \times 10^2$	3.4×10^1
Trickling Filter Humus				
Raw	$1.1 \times 10^6 - 1.9 \times 10^7$	6.6×10^6	$1.4 \times 10^6 - 2.9 \times 10^6$	2.1×10^6
Treated	$2.9 \times 10^2 - 9.6 \times 10^4$	2.2×10^3	$1 \times 10^0 - 2.9 \times 10^2$	2.3×10^1
Mixed Primary/ Trickling Filter Humus				
Raw	$3.1 \times 10^6 - 1.9 \times 10^7$	8.5×10^6	$1.5 \times 10^6 - 4.6 \times 10^6$	2.4×10^6
Treated	$1.0 \times 10^2 - 7.1 \times 10^3$	3.1×10^2	$1 \times 10^0 - 3.4 \times 10^1$	5×10^0

*Counts and Shuckrow (1974). Sludge pH, 12.0; detention time, 0.5 hr.

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TABLE 56. DENSITIES OF INDICATOR BACTERIA IN FOUR TYPES OF SLUDGE BEFORE AND AFTER LIME STABILIZATION TREATMENT*
(Numbers/100 ml)

Sludge Type	Total Coliform Bacteria		Fecal Coliform Bacteria		Fecal Streptococcus Bacteria	
	Range	Mean	Range	Mean	Range	Mean
Primary						
Raw	$2.7 \times 10^8 - 7.2 \times 10^8$	6.0×10^8	$2.7 \times 10^7 - 6.8 \times 10^8$	1.7×10^8	$5.3 \times 10^6 - 1.1 \times 10^7$	8.0×10^6
Treated	$1.7 \times 10^2 - 1.4 \times 10^5$	3.0×10^4	$1.5 \times 10^2 - 4.4 \times 10^3$	1.6×10^3	$1.0 \times 10^2 - 1.4 \times 10^4$	4.2×10^3
Waste Activated						
Raw	$1.3 \times 10^8 - 1.2 \times 10^9$	6.8×10^8	$1.6 \times 10^7 - 2.7 \times 10^7$	2.2×10^7	$4.1 \times 10^5 - 1.6 \times 10^7$	8.3×10^6
Treated	$3.1 \times 10^3 - 3.9 \times 10^5$	2.0×10^5	$3.1 \times 10^2 - 3.0 \times 10^4$	1.5×10^4	$1.4 \times 10^3 - 1.3 \times 10^4$	6.3×10^3
Septage						
Raw	$8.5 \times 10^6 - 3.3 \times 10^8$	1.4×10^8	$4.7 \times 10^6 - 8.5 \times 10^6$	7.1×10^6	$1.6 \times 10^5 - 5.7 \times 10^5$	3.2×10^5
Treated	$8.6 \times 10^1 - 1.7 \times 10^3$	9.1×10^2	$8.6 \times 10^1 - 1.4 \times 10^2$	1.1×10^2	$1.4 \times 10^2 - 4.3 \times 10^2$	2.9×10^2
Anaerobically Digested						
Raw	-	4.6×10^6	$5.6 \times 10^3 - 1.1 \times 10^5$	4.3×10^4	-	4.4×10^4
Treated	-	3.0×10^0	-	5.0×10^2	$5.0 \times 10^1 - 2.1 \times 10^3$	1.3×10^3

*Noland _et al_. (1970). Sludge pH, 12.0 to 12.4; detention time, mixing time plus 0.5 hr, total 1.4 to 4.1 hr.

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TABLE 57. DENSITIES OF INDICATOR BACTERIA IN SEPTAGE BEFORE AND AFTER LIME STABILIZATION TREATMENT*
(MPN/100 ml)

	Fecal Coliform Bacteria		Fecal Streptococcus Bacteria	
	Range	Mean	Range	Mean
Septage (Raw)	$2.4 \times 10^5 - 8.1 \times 10^5$	4.7×10^5	$1.1 \times 10^5 - 7.1 \times 10^6$	8.3×10^5
Septage (Treated)	$2.2 \times 10^2 - 1.3 \times 10^3$	4.2×10^2	$6.1 \times 10^3 - 2.3 \times 10^5$	3.9×10^4

*Feige et al. (1975). Sludge pH, 11.5; detention time, time required to load lime slurry.

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b. pH--The pH level has been related to kill of indicator organisms in a study by Farrell et al. (1974), who looked at density levels of fecal coliform and fecal streptococcus bacteria in a chemically precipitated raw primary sludge before and after lime stabilization to pH 10.5, 11.5 and 12.5 (Table 58).

c. Detention time--In the same study as reported above, Farrell et al. treated primary sludge to pH 11.5 at detention times of 0.5 hr and 24.0 hrs, respectively. Densities of fecal coliform and fecal streptococcus bacteria before and after lime stabilization are reported in Table 59.

d. pH, detention time, and sludge-solids' concentration--Counts and Shuckrow (1974) examined survival of fecal coliform and fecal streptococcus bacteria exposed to a range of pH levels between 11.0 and 12.4 and sampled after detention times of 1 hr and 24 hr, respectively. The laboratory-scale tests were run on sludge at 2.0-percent solids (Table 60) and 4.4-percent solids (Table 61).

Pathogenic Bacteria. The investigators who examined survival of indicator bacteria in sludges subjected to lime stabilization at laboratory or pilot scale have also studied survival of two types of pathogenic bacteria, Salmonella spp. and Pseudomonas aeruginosa. Densities of these bacteria before and after stabilization have been analyzed with respect to variables of sludge type, pH, detention time, and sludge-solids concentration.

a. Sludge type--Using the pilot-scale testing methods described above for their work on indicator bacteria, Counts and Shuckrow (1974) measured densities of Salmonella and Pseudomonas aeruginosa in primary sludge, trickling-filter humus, and mixed primary sludge and trickling-filter humus. Exposed to pH levels of 12.0 to 12.4 for 0.5-hr detention times, the pathogenic bacteria were reduced to below detectable levels in every instance (Table 62).

Noland et al. (1978) studied survival of these same bacteria in primary sludge, waste activated sludge, septage, and anaerobically digested sludge. Samples were raised to pH of 12.0 for detention times of 1.4 to 4.1 hr, depending on the time necessary to raise the sludge to that pH plus 0.5 hr. Results are shown in Table 63. In most cases, pathogenic bacteria were reduced to levels below detection.

b. pH--Farrell et al. (1974) tested survival of Salmonella sp. and Ps. aeruginosa in primary sludge treated to pH levels of 10.5, 11.5, and 12.5 for a detention time of 0.5 hr. Both types of bacteria were reduced to densities that were insignificant or below detection limits at all three pH levels (Table 64); Ps. aeruginosa showed somewhat greater resistance at pH 10.5 than at higher pH levels, but the difference may not be significant.

c. Detention time--In the same study, Farrell et al. compared densities of Salmonella and Ps. aeruginosa in sludge treated to pH

TABLE 58. DENSITIES OF INDICATOR BACTERIA IN PRIMARY SLUDGE BEFORE AND AFTER 0.5 HRS DETENTION AT THREE pH LEVELS*
(Numbers/100 ml)

	Fecal Coliform Bacteria	Fecal Streptococcus Bacteria
Raw sludge	7.2×10^6	4.4×10^5
pH 10.5	5.8×10^6	$> 4.4 \times 10^5$
pH 11.5	2.2×10^3	7.5×10^4
pH 12.5	7.2×10^2	4.0×10^3

*From Farrell et al. (1974). Detention time, 0.5 hr.

TABLE 59. DENSITIES OF INDICATOR BACTERIA IN PRIMARY SLUDGE BEFORE AND AFTER TREATMENT AT pH 11.5 FOR TWO DETENTION TIMES*
(Numbers/100 ml)

	Fecal Coliform Bacteria	Fecal Streptococcus Bacteria
Raw sludge	7.2×10^6	4.4×10^5
0.5 hrs detention	2.2×10^3	7.5×10^4
24.0 hrs detention	7.2×10^2	2.6×10^4

*From Farrell et al. (1974). Sludge pH, 11.5.

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TABLE 60. DENSITIES OF INDICATOR BACTERIA FOLLOWING LIME STABILIZATION OF 2.0-PERCENT-SOLIDS SLUDGE AT DIFFERENT pH AND DETENTION TIMES*
(Numbers/100 ml) (n=2)

Microorganism	Fecal Coliform Bacteria		Fecal Streptococci	
DENSITY IN RAW SLUDGE (pH = 6.0)	8.2×10^6 - 9.5×10^6 (8.8×10^6)		3.2×10^6 - 3.4×10^6 (3.3×10^6)	
Detention Time	One Hour	One Day	One Hour	One Day
pH 11.0				
Range	ND[a] - 5.0×10^3	1.0×10^4 - 1.1×10^4	1.7×10^5 - 1.9×10^5	1.9×10^6 - 6.3×10^6
Mean	7.1×10^1	1.1×10^4	1.8×10^5	3.4×10^6
pH 11.2				
Range	ND - 2.5×10^3	ND	8.0×10^5 - 2.9×10^6	1.9×10^6 - 8.9×10^6
Mean	5.0×10^1	-	1.5×10^6	4.1×10^6
pH 11.4				
Range	ND - 2.5×10^3	5.0×10^3 - 2.6×10^4	6.0×10^4 - 1.6×10^5	4.6×10^5 - 4.6×10^5
Mean	5.0×10^1	1.2×10^4	9.7×10^4	4.6×10^5
pH 11.6				
Range	5.0×10^3 - 1.0×10^4	1.3×10^4 - 2.5×10^4	1.5×10^4 - 7.0×10^4	2.0×10^6 - 1.8×10^7
Mean	7.1×10^3	1.8×10^4	3.2×10^4	6.0×10^6
pH 11.8				
Range	ND	ND - 5.0×10^3	8.5×10^4 - 1.0×10^5	2.0×10^6 - 2.0×10^6
Mean	-	7.1×10^1	9.2×10^4	2.0×10^6
pH 12.0				
Range	1.0×10^4 - 1.3×10^4	2.3×10^4 - 4.8×10^5	1.3×10^3 - 1.3×10^3	1.5×10^6 - 1.7×10^6
Mean	1.1×10^4	1.0×10^5	1.3×10^3	1.6×10^6
pH 12.4				
Range	ND	ND	1.0×10^3 - 1.4×10^3	9.5×10^5 - 1.7×10^6
Mean	-	-	1.2×10^3	1.3×10^6

*From Counts and Shuckrow (1974).

[a] ND= Not detected

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TABLE 61. DENSITIES OF INDICATOR BACTERIA FOLLOWING LIME STABILIZATION OF 4.4-PERCENT-SOLIDS SLUDGE AT DIFFERENT pH AND DETENTION TIMES* (n=2)
(Numbers/100 ml)

Microorganism	Fecal Coliform Bacteria		Fecal Streptococci	
Densities in Raw Sludge (pH = 6.0)	$3.6 \times 10^6 - 4.6 \times 10^6$ (4.1×10^6)		$9.4 \times 10^6 - 1.1 \times 10^7$ (1.0×10^7)	
Detention Time	One Hour	One Day	One Hour	One Day
pH 11.0				
Range	$8.3 \times 10^3 - 9.9 \times 10^3$	$6.5 \times 10^3 - 7.4 \times 10^3$	$1.2 \times 10^7 - 1.5 \times 10^7$	$1.7 \times 10^7 - 1.7 \times 10^7$
Mean	9.1×10^3	6.9×10^3	1.3×10^7	1.7×10^7
pH 11.2				
Range	$6.1 \times 10^3 - 8.5 \times 10^3$	$3.1 \times 10^3 - 3.9 \times 10^3$	$1.9 \times 10^7 - 1.9 \times 10^7$	$1.7 \times 10^7 - 1.8 \times 10^7$
Mean	7.2×10^3	3.5×10^3	1.9×10^7	1.7×10^7
pH 11.4				
Range	$3.7 \times 10^3 - 4.8 \times 10^3$	$7.3 \times 10^3 - 1.1 \times 10^4$	$1.5 \times 10^7 - 1.5 \times 10^7$	$1.9 \times 10^7 - 1.9 \times 10^7$
Mean	4.2×10^3	9.1×10^3	1.5×10^7	1.9×10^7
pH 11.6				
Range	$1.1 \times 10^4 - 1.2 \times 10^4$	$5.8 \times 10^3 - 8.6 \times 10^3$	$1.0 \times 10^6 - 1.0 \times 10^6$	$1.9 \times 10^7 - 1.9 \times 10^7$
Mean	1.2×10^4	7.1×10^3	1.0×10^6	1.9×10^7
pH 11.8				
Range	$7.9 \times 10^3 - 1.5 \times 10^4$	$1.2 \times 10^4 - 1.3 \times 10^4$	$1.9 \times 10^6 - 1.9 \times 10^6$	$1.9 \times 10^6 - 2.0 \times 10^6$
Mean	1.1×10^4	1.3×10^4	1.9×10^6	1.9×10^6
pH 12.0				
Range	$5.1 \times 10^3 - 8.4 \times 10^3$	$2.6 \times 10^3 - 5.0 \times 10^3$	$7.4 \times 10^4 - 7.7 \times 10^4$	$5.7 \times 10^5 - 7.0 \times 10^5$
Mean	6.6×10^3	3.6×10^3	7.5×10^4	6.3×10^5
pH 12.4				
Range	$6.1 \times 10^3 - 1.2 \times 10^4$	$1.6 \times 10^4 - 1.8 \times 10^4$	$1.6 \times 10^5 - 1.8 \times 10^5$	$5.8 \times 10^5 - 5.9 \times 10^5$
Mean	8.6×10^3	1.7×10^4	1.7×10^5	5.9×10^5

*From Counts and Shuckrow (1974).

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TABLE 62. DENSITIES OF PATHOGENIC BACTERIA IN THREE TYPES OF SLUDGE BEFORE AND AFTER LIME-STABILIZATION TREATMENT*

(MPN/100 ml)

Sludge Type	Pseudomonas aeruginosa		Salmonella	
	Range	Mean	Range	Mean
Primary				
Raw	$2.0 \times 10^3 - 8.2 \times 10^4$	1.6×10^4	$9.7 \times 10^2 - 2.0 \times 10^3$	1.4×10^3
Treated	-	ND[a]	-	ND
Trickling Filter Humus				
Raw	$1.5 \times 10^3 - 9.4 \times 10^4$	1.2×10^4	$7.4 \times 10^2 - 5.8 \times 10^3$	2.1×10^3
Treated	-	ND	-	ND
Mixed Primary/ Trickling Filter				
Raw	$6.5 \times 10^3 - 2.0 \times 10^4$	1.2×10^4	$1.2 \times 10^3 - 2.2 \times 10^3$	1.6×10^3
Treated	-	ND	-	ND

*From Counts and Shuckrow (1974). Sludge pH, 12.0; Detention time, 0.5 hr.

[a] ND= Not detected

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TABLE 63. DENSITIES OF PATHOGENIC BACTERIA IN FOUR TYPES OF SLUDGE BEFORE AND AFTER LIME-STABILIZATION TREATMENT*
(MPN/100 ml)

	Pseudomonas aeruginosa		Salmonella	
Sludge Type	Range	Mean	Range	Mean
Primary				
Raw	15 - 90	40	2 - 49	13
Treated	-	< 1	-	< 1
Waste Activated				
Raw	$7.4 \times 10^1 - 8.9 \times 10^3$	4.5×10^3	2 - 7	5
Treated	3 - 24	12	-	< 3
Septage				
Raw	$7. \times 10^0 - 9.9 \times 10^2$	3.6×10^2	1 - 4	3
Treated	-	< 1.3	-	< 1.3
Anaerobically Digested				
Raw	1 - 39	7	4 - 5	1
Treated	-	< 1	-	< 1

*From Noland *et al.* (1978). Sludge pH, 12.0 to 12.4; detention time, mixing time *plus* 0.5 hr, total 1.4 to 4.1 hr.

TABLE 64. DENSITIES OF PATHOGENIC BACTERIA IN PRIMARY SLUDGE BEFORE AND AFTER 0.5 HRS DETENTION AT THREE pH LEVELS*
(MPN/100 ml)

	Salmonella spp.	*Pseudomonas aeruginosa*
Density in raw sludge	> 220	220
pH 10.5	0.7	1.8
pH 11.5	< 0.6	0.7
pH 12.5	< 0.6	< 0.6

*From Farrell *et al.* (1974). Detention time, 0.5 hr.

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11.5 and tested at 0.5 hr and 24.0 hr, respectively. Again, the pathogenic bacteria were reduced to insignificant or undetectable levels. The 24-hr samples gave no indication of regrowth (Table 65).

d. pH, detention time, and sludge-solids' concentration--As described above for laboratory-scale work on indicator bacteria, Counts and Shuckrow (1974) examined survival of *Salmonella* sp. and *Pseudomonas aeruginosa* bacteria exposed to a range of pH levels between 11.0 and 12.4 and sampled after detention time of 1 hr and 24 hr, respectively. The tests were run on sludges at 2.0-percent solids (Table 66) and 4.4-percent solids (Table 67).

Viruses. Sattar *et al*. (1976), in a study of virus removals conducted at laboratory scale, injected 1-liter aliquots of sewage with an average of 3.3×10^7 PFU of poliovirus type 1 (Sabin). A lime slurry was added, drop by drop, until a pH of 11.5 was achieved. The sewage sample was settled and centrifuged, and then the sludge, supernate from settling, and centrate were tested for surviving polio viruses. Viruses were not detectable in the supernatant or in the centrate. An average of 1.3×10^3 PFU of virus was found in the sludge portion. This represents an inactivation of greater than 4 logs with respect to the initial count of viruses present in the sewage.

Parasites. In pilot-scale tests run by Noland *et al*. (1978), using high-lime methods described above for their work on indicator and pathogenic bacteria, the high pH of the sludge had little effect on the viability of parasites in the limed sludges. Viable parasites were found in both limed and unlimed samples. Numbers were reduced in the limed samples (Noland, personal communication). All sludges tested showed presence of *Toxocara*,

In the Reimers *et al*. (1980) tests, they observed a decrease in viable *Ascaris* eggs from swine intestinal material after lime addition, but some of their tests used higher concentrations of lime than are normally used for lime conditioning and results were not always consistent.

At a dosage of 76-percent CaO, (about three times dosages used for lime stabilization), and pH between 12.9 and 13.3 after 1 hr, some reductions were noted in samples held for 1 hr after lime addition. Viable eggs in two aerobically digested sludge portions held at aerobic conditions after lime addition decreased by about 1 log (for a sample digested at 28°C) and about 1.8 logs (for a sample digested at 35°C). Samples of other sludges (limed primary sludge and lime aerobically digested sludges) held under anaerobic conditions were not analyzed after 1 hr. After 5 days (the next sampling), all the samples receiving a dosage of 76 percent showed reductions exceeding 0.7 logs, except for the sample digested at 35°C and held at anaerobic conditions after lime addition. Reduction for that sample was only about 0.1 log.

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TABLE 65. DENSITIES OF PATHOGENIC BACTERIA IN PRIMARY SLUDGE BEFORE AND AFTER TREATMENT AT pH 11.5 FOR TWO DETENTION TIMES* (MPN/100 ml)

	Salmonella spp.	*Pseudomonas aeruginosa*
Density in raw sludge	> 220	220
0.5 hrs detention	< 0.6	0.7
24.0 hrs detention	< 0.6	< 0.6

*From Farrell *et al*. (1974). Sludge pH, 11.5.

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TABLE 66. DENSITIES OF PATHOGENIC BACTERIA FOLLOWING LIME STABILIZATION OF 2.0-PERCENT-SOLIDS SLUDGE AT DIFFERENT pH AND DETENTION TIMES (n=2)* (MPN/100 ml)

Microorganism	Salmonella spp.		Pseudomonas aeruginosa	
Densities in Raw Sludge (pH = 6.0)	130 - 230 (176)		155 - 260 (201)	
Detention Time	One Hour	One Day	One Hour	One Day
pH 11.0				
Range	39 - 65	23 - 55	18 - 37	ND
Mean	50	35	26	-
pH 11.2				
Range	20 - 23	34 - 55	9 - 9	ND
Mean	21	43	9	-
pH 11.4				
Range	ND[a] - 10	10 - 23	ND	ND
Mean	3	15	-	-
pH 11.6				
Range	ND	23 - 55	9 - 10	ND
Mean	-	35	9	-
pH 11.8				
Range	23 - 23	10 - 10	10 - 10	ND - 10
Mean	23	10	10	3
pH 12.0				
Range	23 - 23	65 - 170	ND	ND
Mean	23	105	-	-
pH 12.4				
Range	10 - 23	ND - 23	ND	ND
Mean	15	5	-	-

*Counts and Shuckrow (1974).

[a] ND= Not detected

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TABLE 67. DENSITIES OF PATHOGENIC BACTERIA FOLLOWING LIME STABILIZATION OF 4.4-PERCENT-SOLIDS SLUDGE AT DIFFERENT pH AND DETENTION TIMES* (n=2) (MPN/100 ml)

Microorganism	Salmonella		Pseudomonas aeruginosa	
Densities in Raw Sludge (pH = 6.0)	1300 - 2500 1800		636 - 1950 1120	
Detention Time	One Hour	One Day	One Hour	One Day
pH 11.0				
Range	50 - 245	77 - 77	29 - 33	318 - 1590
Mean	111	77	31	711
pH 11.2				
Range	77 - 77	59 - 100	64 - 64	2 - 2
Mean	77	77	64	2
pH 11.4				
Range	5 - 73	59 - 127	21 - 22	14 - 418
Mean	19	87	22	77
pH 11.6				
Range	10 - 23	12 - 50	3 - 4	1 - 2
Mean	15	24	3	1
pH 11.8				
Range	22 - 59	8 - 50	1 - 1	2 - 2
Mean	36	20	1	2
pH 12.0				
Range	1 - 2	6 - 6	ND[a]	ND - 2
Mean	1	6	-	1
pH 12.4				
Range	3 - 4	9 - 15	ND	6 - 6
Mean	3	12	-	6

*Counts and Shuckrow (1974).

[a] ND= Not detected

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At a dosage of 8-percent CaO and pH of 8.7 to 12.5 after 1 hr, no reduction in viable Ascaris eggs was found 1 hr after lime addition. At 5 days, reduction for the raw sludge was .05 logs. Reductions in the aerobically digested sludges ranged from none to 0.3 logs.

Discussion

Lime stabilization can effect significant reductions in levels of some indicator and pathogenic bacteria and, possibly, of poliovirus. Its effectiveness has been shown to be contingent upon the pH achieved in the stabilization protocol. It appears that different classes of bacteria respond differently to the increasing levels of pH achieved in the process. The "decay" in pH level that occurs after the initial exposure and minimum contact time can create an environment favorable to regrowth of some bacteria.

Figure 20 shows the response to pH exhibited by two types of indicator bacteria and two genera of pathogenic bacteria in primary sludge treated by lime stabilization. Densities of the indicator fecal coliform and fecal streptococcus bacteria are seen to be unaffected at pH 10.5 but reduced by increasing orders of magnitude at pH levels of 11.5 and 12.5.

Fecal streptococcus bacteria appear to be less sensitive to the high-pH environment than fecal coliform bacteria, showing only moderate reductions in density--of less than 1 log--at pH 11.5. The pathogenic Salmonella spp. and Ps. aeruginosa are both reduced in densities by two or more orders of magnitude at pH 10.5 and above.

The effect of pH decay over time is not well established. The data of Farrell et al. (1974) indicate that lime treatment to pH 11.5 may remain effective for up to 24 hr in reducing densities of indicator and pathogenic bacteria (Figure 21). Other researchers have found that fecal streptococcus bacteria, in particular, exhibit marked regrowth within 24 hr, even when initial pH is raised as high as 12.4 (Figures 22 and 23).

Figures 22 to 25 present a graphical summary of the effectiveness of lime stabilization in killing four types of bacterial organisms present in municipal wastewater sludge. Adapted from data of Counts and Shuckrow (1974), the figures show the influence of initial pH, pH decay over 24 hr, and sludge-solids' concentration. The following conclusions can be made:

a. At pH 11.0 and above, fecal coliform bacteria are reduced in density by 2 to 7 logs, regardless of sludge-solids concentration (Figures 22 and 23).

b. Fecal streptococcus bacteria are more resistant than fecal coliform bacteria to high pH, showing consistent reductions on the order of 1 to 3 logs only at pH 11.6 and above (Figures 22 and 23). Moreover, the fecal streptococcus bacteria are able to respond quickly to decaying pH, showing regrowth to near-original densities or greater within 24 hr.

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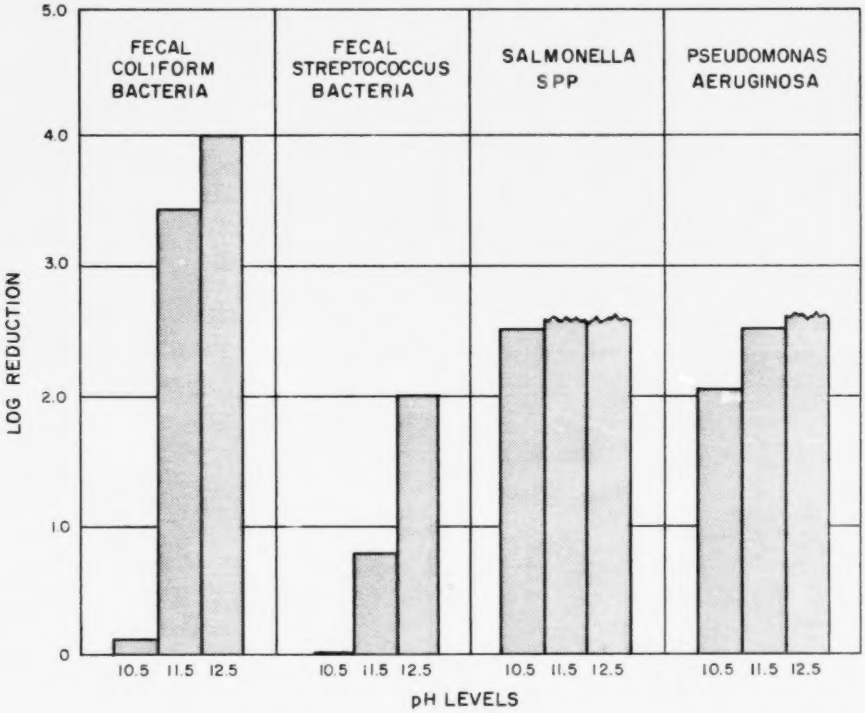

FIGURE 20. EFFECTS OF THREE pH LEVELS ON INDICATOR AND PATHOGENIC BACTERIA IN SLUDGE AT 5.0 PERCENT SOLIDS CONCENTRATION (0.5 HR. DETENTION TIME) *

* FROM FARRELL et al. (1974)

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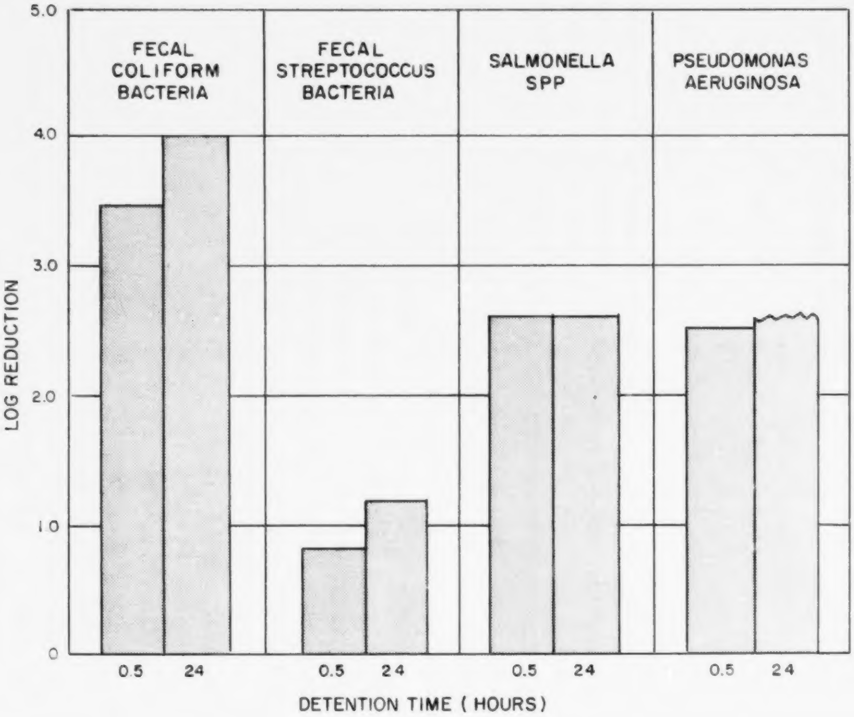

FIGURE 21. EFFECTS OF DETENTION TIME ON INDICATOR AND PATHOGENIC BACTERIA IN PRIMARY SLUDGE TREATED TO pH 11.5. *

* FROM DATA OF FARRELL et al. (1974)

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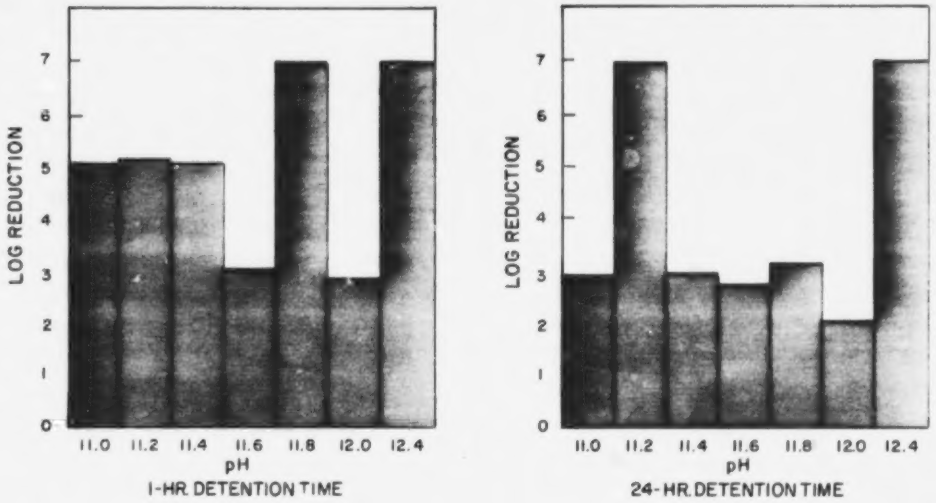
FECAL COLIFORM BACTERIA



FECAL STREPTOCOCCUS BACTERIA

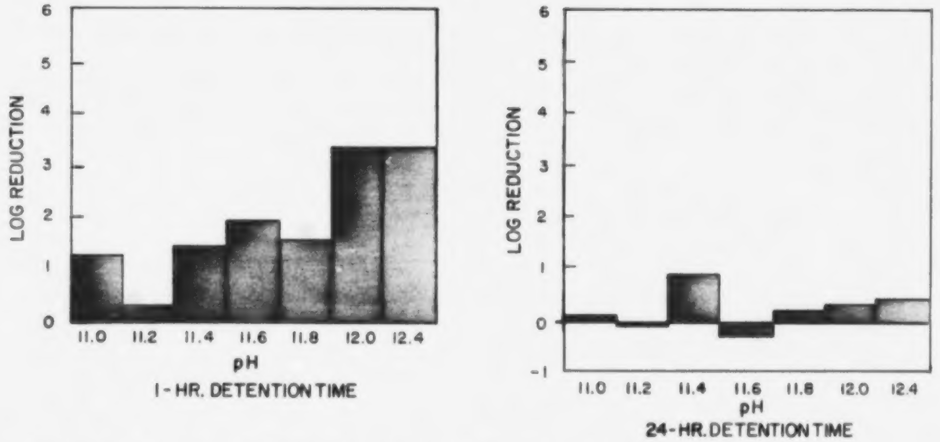


FIGURE 22. REDUCTIONS IN FECAL COLIFORM AND STREPTOCOCCUS BACTERIA IN 2.0 PERCENT SOLIDS SLUDGE TREATED BY LIME STABILIZATION.*

* FROM DATA OF COUNTS AND SHUCKROW (1974)

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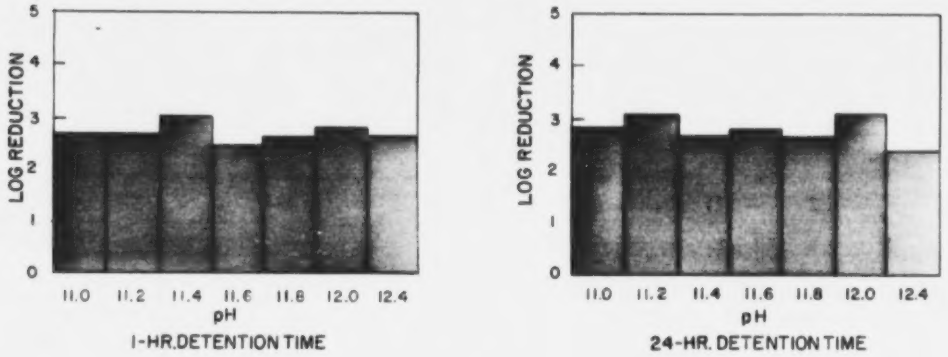
FECAL COLIFORM BACTERIA



FECAL STREPTOCOCCUS BACTERIA

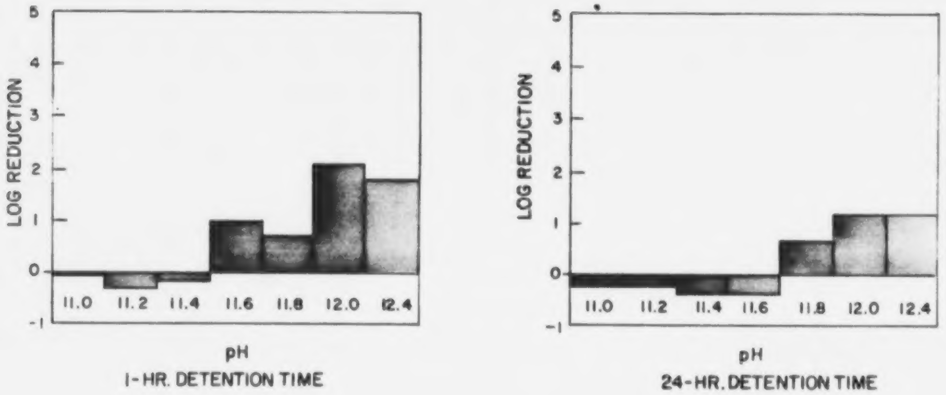


FIGURE 23. REDUCTIONS IN FECAL COLIFORM AND FECAL STREPTOCOCCUS BACTERIA IN 4.4 PERCENT SOLIDS SLUDGE TREATED BY LIME STABILIZATION.*

* FROM DATA OF COUNTS AND SHUCKROW (1974)

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c. At pH 11.0 and above, pathogenic *Salmonella* spp. and *Pseudomonas aeruginosa* appear to be reduced consistently. Neither type of pathogenic organisms shows a pronounced response to increasing pH above 11.0; their thresholds of sensitivity appear to lie well below pH 11.0 (Figure 24 and 25), as was also indicated by data of Farrell *et al.*, as shown in Figure 20.

d. Reductions in densities of the pathogenic bacteria tend to be relatively modest. In most cases, however, this is because initial densities of these organisms are so low. A reduction of less than one order of magnitude may put the density of either *Salmonella* or *Ps. aeruginosa* close to its limit of detection (see Tables 62 and 63, for example).

e. The pathogenic bacteria *Salmonella* and *Ps. aeruginosa* show no apparent tendency to regrow within 24 hr (Figures 24 and 25) when exposed to pH levels of 11.0 and above.

f. Increased sludge-solids concentration appears to moderate the bacterial response to pH. Comparing Figures 22 and 23, for example, it can be seen that reductions of fecal coliform are far more consistent--in fact, predictable--in sludge at 4.4-percent solids concentration than in sludge at 2.0-percent solids concentration.

The same is true for response of fecal streptococcus bacteria; moreover, regrowth of that type of bacteria is dampened in sludge at higher solids concentration.

Sludge type, in most cases, appears to have no impact on the effectiveness of lime stabilization (Figure 26, from data of Counts and Shuckrow (1974) and Noland *et al.* (1978)). The indicator fecal coliform and fecal streptococcus bacteria are, however, seen to be reduced by significantly lower orders in anaerobically digested sludge than in raw sludge or septage. This may be due to the fact that only hardier strains of these bacteria have survived the conditions of digestion and that these strains are resistant also to the high-pH environment.

The extremely limited data base prevents any valid comparison between results obtained at laboratory-scale and results obtained at pilot-scale. The data are consistent, however, in showing reductions of fecal coliform and fecal streptococcus bacteria by more than two orders of magnitude at pH 12.0 and above.

Both fecal coliform and fecal streptococcus bacteria appear to be suitable indicators for survival of the pathogens *Salmonella* spp. and *Ps. aeruginosa*. The thresholds of sensitivity for both indicators are higher than that for either pathogen. Fecal streptococcus bacteria could be considered a more conservative indicator, but its pronounced capability for regrowth does not appear to be mimicked by the pathogens.

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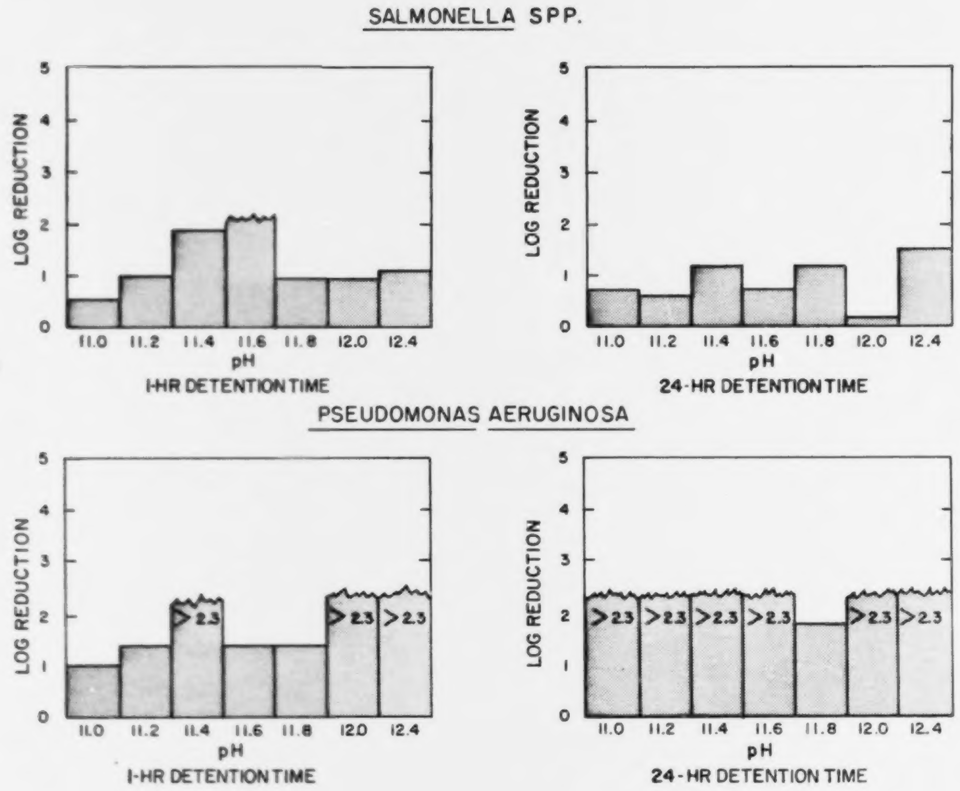

FIGURE 24. REDUCTIONS IN SALMONELLA SPP. AND PSEUDOMONAS AERUGINOSA IN 2.0 PER CENT SOLIDS SLUDGE TREATED BY LIME STABILIZATION.*

* FROM DATA OF COUNTS AND SHUCKROW (1974)

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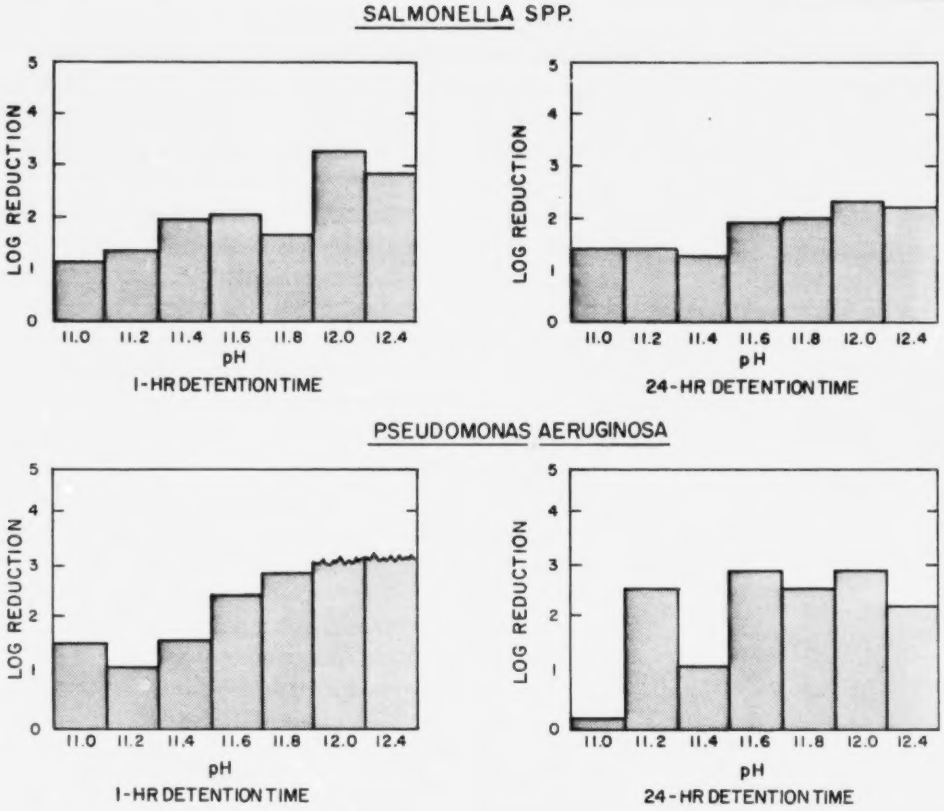

FIGURE 25. REDUCTIONS IN SALMONELLA SPP. AND PSEUDOMONAS AERUGINOSA IN 4.4 PER CENT SOLIDS SLUDGE TREATED BY LIME STABILIZATION. *

* FROM DATA OF COUNTS AND SHUCKROW (1974)

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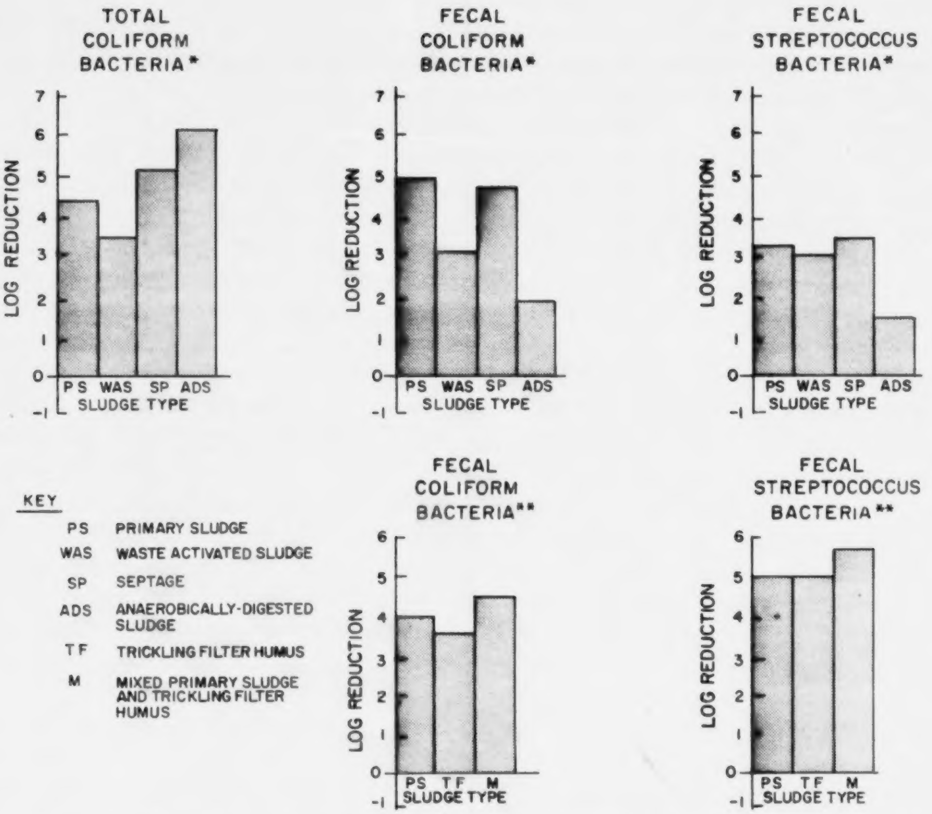

FIGURE 26. REDUCTIONS OF INDICATOR BACTERIA IN SEVERAL TYPES OF SLUDGE TREATED BY LIME STABILIZATION TO pH 12.0 OR GREATER.

* FROM DATA OF NOLAND et al. (1978)
** FROM DATA OF COUNTS AND SHUCKROW (1974)

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Although data are scarce, it appears that lime stabilization of sludge as defined in the EPA *Criteria* is ineffective in achieving reductions of viable *Ascaris* parasites from the intestinal material. The process appears effective in inactivating polio virus by 4 logs or more and in reducing numbers of *Toxocara* ova.

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SECTION 5

CONVENTIONAL SLUDGE DEWATERING PROCESSES

The effectiveness of air drying and sand bed dewatering, sludge storage/lagooning, and sludge conditioning/mechanical dewatering in reducing levels of indicator and pathogenic organisms, enteroviruses and parasites are evaluated in this section.

The organization of each subsection is similar to that described for Section 4, with an introduction, results and discussion.

DRYING BEDS

Introduction

Description of Process. Sand drying beds are the most commonly used type of drying beds, although paved beds (with drainage) are also used. Sand drying beds are commonly used to dewater digested, rather than raw sludge. The sludge is placed in a layer 20 to 30 cm thick and allowed to dry. Beds may be exposed to air, or enclosed in a greenhouse-type structure. The sludge cake is removed from the beds at approximately 60 percent moisture (40 percent solids) after 10-15 days under favorable conditions.

In 40 CFR 257, air drying is designated as a "Process to Significantly Reduce Pathogens". Air drying is defined as follows:

> "Liquid sludge is allowed to drain and/or dry on undrained sand beds, or paved or unpaved basins in which sludge is at a depth of nine inches (22.5 cm). A minimum of three months is needed, two months of which temperatures average on a daily basis above 0°C."

It should be noted that while temperature, depth of sludge, and drying time are specified in 40 CFR 257, a final solids content is not delineated.

Operating Parameters. Parameters that may vary in drying beds are: type of sludge, the method that was used to stabilize the sludge prior to dewatering, the temperature at which the sludge is dried, whether the beds are covered or not, how moisture is removed from the settled sludge and the length of time allowed for dewatering. All of these factors will influence the level of solids encountered in the cake, as well as the resultant levels of pathogenic and indicator organisms after drying.

Data Base Available. All forms of sludge drying processes will be considered in this review with notations made as to the type of bed utilized. Little information was found in this review that followed the reduction of indicator bacteria, pathogenic organisms, viruses and parasites through drying in full-scale plants. The conditions used in laboratory scale experiments were not typical of any one full-scale drying process. For example, studies by Nath and Johnson (1979) and Ward and Ashley (1977b) examine the effects of evaporation of sludge on the survival of enteroviruses under temperature and sludge depth conditions that would not occur at full-scale plants. The general effect of evaporation would be seen, however, in a full-scale plant, and extrapolations to full-scale conditions are of interest.

The following summary briefly describes the articles reviewed in this section:

- Baxter *et al*. (1980) -- Added anaerobically digested primary sludge to earthen basins to a depth of 0.6 m in a series of four experiments designed to investigate effects of outdoor temperature on *Ascaris*, f2 virus, total coliforms, fecal coliforms, and *Salmonella*. The f2 virus and *Ascaris* ova had been seeded into the sludge before drying. The sludge was sampled every three weeks in these experiments.

- Bond (1958)--Examined digested sludge from the Tampa Municipal Sewage Disposal Plant which had been distributed to drying beds for up to 21 days. (The Tampa plant provided primary treatment. Sludge was digested for 30 days at 27°C to 32°C.) Ova of *Ascaris* sp. were enumerated.

- Brandon and Neuhauser (1977)--Subjected both digested and raw sludge to drying at a depth of 5 cm (2 in) for a maximum of 4 days. Survivals of coliform bacteria, fecal streptococci, *Salmonella* sp. and *Aspergillus flavus* were followed during this study.

- Cram (1943)--Studied the effects of drying on seeded ova of *Ascaris lumbricoides* and the dog hookworm, *Ancylostoma caninum*. Imhoff-tank sludge was dried, at an unspecified depth, for up to 189 days.

- Feige *et al*. (1975)--In this study, septage that had been limed to pH levels of 10.5, 11 and 11.5 was dewatered on sand beds. The survivals of fecal coliform and fecal streptococcus bacteria were examined.

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- Fuller and Litsky (1950)--In this laboratory-scale study, densities of *Escherichia coli* in digested sludge that had been dried for 80 days was examined. A comparison of survival at 22°C and at 37°C at a sludge depth of 15 cm (6 in) was made.

- Heukelekian and Albanese (1956)--The survival of tubercle bacteria (*Mycobacterium* sp.) in a laboratory-scale drying bed was studied in this report. Tubercle bacteria was seeded into sludge that was subsequently digested and then dried, at a depth of 30 cm (12 in), for up to 25 days.

- Jones *et al*. (1947)--Digested sludge was dried, at a depth of 18 to 25 cm (7 to 10 in), for up to 23 days at a temperature of 24-29°C. Survival of *Schistosoma japonicum* was followed in this laboratory-scale experiment.

- Keller and Hide (1951)--The viability of *Ascaris lumbricoides* ova in digested and drying sludge was the subject of this experiment. The digested sludge was dried for up to 42 days in the warm climate of Johannesburg, South Africa.

- Nath and Johnson (1979)--Compared the effect of dewatering primary sludge on seeded poliovirus, type 2 at 22.5°C to that at 37°C. Seeded sludge was dried for a maximum of 25 hr on a laboratory scale.

- Newton *et al*. (1948)--Examined survival of *Schistosoma japonicum* under conditions identical to that of Jones *et al*. (1947), except that temperatures of 15-24°C and 29-32°C were used.

- Reimers *et al*. (1980)--Collected sludge samples from domestic WWTPs in southern U.S., and analyzed them for parasites. The parasites investigated are *Ascaris*, *Toxocara*, *Trichuris trichiura* and *Trichuris vulpis*. Samples were taken of raw sludges before digestion and of dried sludge after aerobic or anaerobic digestion. The samples were taken from eight plants with aerobic digesters and five plants with anaerobic digesters. Data were not reported for the sludge immediately before drying (after stabilization).

- Ward and Ashley (1977b)--Moisture was evaporated from raw sludge that had been seeded with poliovirus type 1 CHAT, coxsackie virus B1, and reovirus. All samples were held at 21°C for 4 or 11 days.

- Wellings *et al*. (1976)--Enumerated enterovirus in a sludge sample that had been dried on a sand bed in Florida for 13 days. No other information was presented on the sludge history.

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Results

Indicator Bacteria. At a sludge depth of 15 cm, and a temperature of 22°C, a greater than 3-log reduction of coliform bacteria was reported by Fuller and Litsky (1950) after 80 days of sand-bed drying (Table 68). The authors compared reduction of coliform bacteria at 37°C and 22°C, and found a faster rate of reduction at the higher temperature. After 28 days at 37°C, a 2.98-log reduction was found, while at 22°C, a 1.2-log reduction occurred. In this experiment, greater than 95-percent solids was achieved within 7 days of drying at both temperatures studied.

In the same experiment, levels of *Escherichia coli* were examined. While exact quantification of *E. coli* was not made, the highest dilution from which *E. coli* was recovered was reported. *E. coli* was not recovered after 21 days at 22°C, while at 37°C, *E. coli* was recovered up to 56 days. From this study, it was concluded that *E. coli* (as opposed to coliform bacteria) survived longer at 37°C than at 22°C (Fuller and Litsky, 1950).

Baxter *et al*. (1978) found that the potential exists for regrowth of fecal and total coliform bacteria in sludge dried in outdoor earthen basins. Because of regrowth, fecal coliform bacteria were enumerated for 73 weeks in one of their four experiments. In the three others, fecal coliform bacteria were not found after 27 weeks.

Fecal coliform bacteria were reduced 1.3 logs in lime-conditioned septage at pH 11 when the septage was applied to sand beds and dried 16 days (Table 69, Feige *et al*., 1975). Reductions at pH 10.5 and 11.5 were similar. Similar results were found in two additional experimental runs conducted for shorter time periods, although in run 4, fecal coliform bacteria increased at pH 11 after 11 days.

Fecal streptococcus bacteria, enumerated in the same experiment, appear to be more resistant to the combination of liming and drying than fecal coliform bacteria. After 16 days, a 1.86-log reduction occurred at pH 11.5. a 0.07-log reduction at pH 11.0, and a 0.26-log reduction at pH 10.5 (Table 70).

Pathogenic Bacteria. The density of *Salmonella* seeded into sterile digested sludge was reduced by approximately 6 logs (from 10^9 to 10^3 bacteria/g) after 4 days of drying at 15-38°C (Brandon and Neuhauser, 1977). In a similar experiment, conidia of *Aspergillus flavus* were reduced by more than 2 logs, from approximately $5x10^5$ conidia/g to 10^3/g.

During this experiment, solids content of greater than 80 percent were achieved. In a subsequent, but similar experiment utilizing non-sterilized raw and digested sludge whose data were not presented, viable coliforms were found after 3 weeks and fecal streptococci were found after 5 weeks (Brandon and Neuhauser, 1977).

Salmonella organisms can regrow in sludge drying beds (Baxter *et al*., 1980). In an experiment where regrowth occurred, *Salmonella* were found in

TABLE 68. REDUCTION OF COLIFORM BACTERIA IN ANAEROBICALLY DIGESTED SLUDGE APPLIED TO LABORATORY SCALE DRYING BEDS*

Drying Time (days)	37°C			22°C		
	% Solids	Coliform Bacteria MPN/GDW	Log Reductions	% Solids	Coliform Bacteria MPN/GDW	Log Reductions
1	37	1.9×10^7		36	2.2×10^7	
7	97.2	2.2×10^5	1.94	96	5.1×10^6	0.63
28	98.4	2.0×10^4	2.98	96.7	1.4×10^6	1.20
56	98.4	2.0×10^3	3.98	96.7	7.0×10^3	3.50
70	98.4	1.5×10^3	4.10	96.7	5.0×10^3	3.64
80	98.4	8.0×10^2	4.38	96.7	5.0×10^3	3.64

*Fuller and Litsky (1950). Counts were converted from per gram wet weight to per gram dry weight based on percent solids.

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TABLE 69. DENSITIES OF FECAL COLIFORM BACTERIA IN LIMED SEPTAGE APPLIED TO SAND BEDS*

			RUN 2			
	pH 11.5	% Solid	pH 11.0	% Solid	pH 10.5	% Solid
day 0**	$<1 \times 10^3$	4.5	1×10^3	4.5	$<1 \times 10^3$	4.5
day 1**	$<3 \times 10^2$	3.0	$<4 \times 10^2$	3.0	$<4 \times 10^2$	3.5
day 5	<10	10	<50	13	<50	17
day 12	<10	25	<50	23.8	<50	21
day 16	<10	27	<50	26.5	<10	30
			RUN 3			
	pH 11.5		pH 11.0		pH 10.5	
day 0**	$<5 \times 10^2$		$<2 \times 10^3$		5×10^3	
day 1**	$<5 \times 10^2$		$<3 \times 10^2$		6.6×10^3	
day 5	<10		<10		1.5×10^3	
			RUN 4			
	pH 11.5		pH 11.0		pH 10.5	
day 0**	$<2 \times 10^2$		$<2 \times 10^2$		2.7×10^3	
day 1**	$<1 \times 10^2$		$<2 \times 10^2$		$<6.6 \times 10^2$	
day 8	<10		1.2×10^3		7.5×10^2	
day 11	<10		3.5×10^2		10	

*Feige _et al_. (1975). Densities in MPN/GDW.

**Counts per 100 ml converted to counts/GDW.

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TABLE 70. DENSITIES OF FECAL STREPTOCOCCI IN LIMED SEPTAGE APPLIED TO SAND BEDS* MPN PER GRAM

Days	pH 11.5	% Solids	pH 11.0	% Solids	pH 10.5	% Solids
RUN 2						
0**	2x10^{5}	4.5	1.4x10^{6}	4.5	2.7x10^{6}	4.5
1	6.0x10^{5}	3.0	2.9x10^{6}	3.0	4.7x10^{6}1	3.5
5	2.9x10^{4}	10	1.0x10^{4}	13	3.0x10^{5}	17
12	5.0x10	25	4.1x10^{5}	23.8	1.2x10^{5}	21
16	2.7x10^{3}	27	1.2x10^{6}	26.5	1.5x10^{6}	30
RUN 3						
	pH 11.5		pH 11.0		pH 10.5	
0**	1.3x10^{4}		4.3x10^{5}		5x10^{6}	
1**	1.3x10^{4}		3.7x10^{5}		1.2x10^{7}	
5	7x10^{3}		1.1x10^{5}		2.8x10^{6}	
RUN 4						
	pH 11.5		pH 11.0		pH 10.5	
0**	4.4x10^{4}		7.3x10^{4}		2.7x10^{5}	
1**	2.6x10^{4}		2.7x10^{4}		1.5x10^{5}	
8	<50		<50		2.3x10^{4}	
11	<50		350		4.8x10^{5}	

*Feige _et al_. (1975). Densities in MPN/GDW.

**Counts per 100 ml converted to counts/GDW.

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sludge samples dried for 67 weeks in the beds. In this experiment, *Salmonella* were detected at low levels (less than 10 organisms per GDW) until the 30th week of drying, when approximately 10/GDW were enumerated. In three other beds operated at different times of the year, no *Salmonella* were found after 15 weeks.

Tubercle bacteria (*Mycobacterium* sp.) seeded into anaerobically digested sludge were found to be resistant to drying (Heukelekian *et al.*, 1956). 4.2×10^6 tubercle bacteria/GDW were still present after 25 days of drying, a reduction of 0.12 log (Table 71). Muller (1959) also found tubercle bacteria to be resistant to drying. In 100 samples collected after 14 days of drying, 71 percent were positive for *Mycobacterium* sp., and, after 10 days, 44 percent of 100 samples were positive. No initial density data were reported for raw sludge prior to drying.

Viruses. Two laboratory-scale experiments have been conducted examining the effects of evaporation on virus survival (Ward and Ashley, 1977b; Nath and Johnson, 1979, Table 72). Both of these studies utilized seeded enterovirus and were conducted in undrained containers over limited time periods (24 hr to 11 days). Both studies concluded that inactivation of enterovirus was affected by the solids level in the dried sludges, which ranged from 55 to 83 percent solids. In control containers, where no evaporation occurred, no reduction in viral titers was seen.

Nath and Johnson (1979) compared reduction of poliovirus type 2 in sludge dried at 22°C and 37°C for a maximum of 24 hours. Table 72 presents their results.

At about 55-percent solids, at both 22° and 37°C, a breakpoint in the rate of inactivation resulted in the loss of all detectable virus (3.0 log) within 1.5 hr. Temperature of drying increased the rate of evaporation and of die-off of poliovirus in this study (Nath and Johnson, 1979).

Ward and Ashley (1977b), in a more elaborate experiment and somewhat longer term (11 days), examined the effect of dewatering by evaporation on viruses. They eliminated time and temperature as factors contributing to die-off of enteroviruses by storing all dried sludge samples at specified solids levels for both 4 days and 11 days at 21°C. Lower titers were generally found after 11 days storage irrespective of solids levels (Figure 27).

A dramatic decrease in viral titer between 65 and 85 percent solids (Figure 27) was seen for all viruses studied. Poliovirus and reovirus were reduced after 11 days by only 1 log at 65 and 40 percent solids, respectively. After 11 days at 83-percent solids (poliovirus) and 94-percent solids (reovirus), titers of both viruses had been reduced by greater than 4 logs.

Data collected by Baxter *et al.* (1980) showed no correlation between percent-solids and rate-of-reduction of f2 bacteriophage outdoors in earthen basins (Figure 28). In their experiment, the most rapid reduction in viral numbers occurred with solids content of less than 16 percent. In all four experiments, all f2 bacteriophage were inactivated within 19 weeks.

TABLE 71. SURVIVAL OF TUBERCLE BACTERIA (MYCOBACTERIUM SP.) DURING SLUDGE DRYING*

Days Drying	Organisms	Log Reduction
1	5.5×10^6	
3	2.9×10^6	0.28
10	6.3×10^6	-0.06
25	4.2×10^6	0.12

*Heukelekian and Albanese, 1956. No./GDW.

TABLE 72. REDUCTION OF SEEDED POLIVIRUS, TYPE 2 DURING EVAPORATION OF PRIMARY SLUDGE*

	22.5°C			37°C		
Time (hours)	% Solids	Total PFU**	Log Reduction	% Solids	Total PFU**	Log Reduction
0	2.27	8.45×10^6	-	2.27	9.13×10^6	0
12	2.58	5.96×10^6	0.15	2.63	7.31×10^6	0.10
18	4.80	2.42×10^6	0.54	43.3	7.22×10^5	1.10
19	-	-	-	79.8	9.0×10^2	4.01
20	-	-	-	92.5	$<1.0 \times 10^2$	>4.96
23	41.6	2.79×10^5	1.48			
23.5	55	9.02×10^4	1.97			
24	71.9	$<1.0 \times 10^2$	>4.93			
25	90.9	$<1.0 \times 10^2$	>4.93			

* Nath and Johnson, 1979.

** Total virus in a 50-ml sample.

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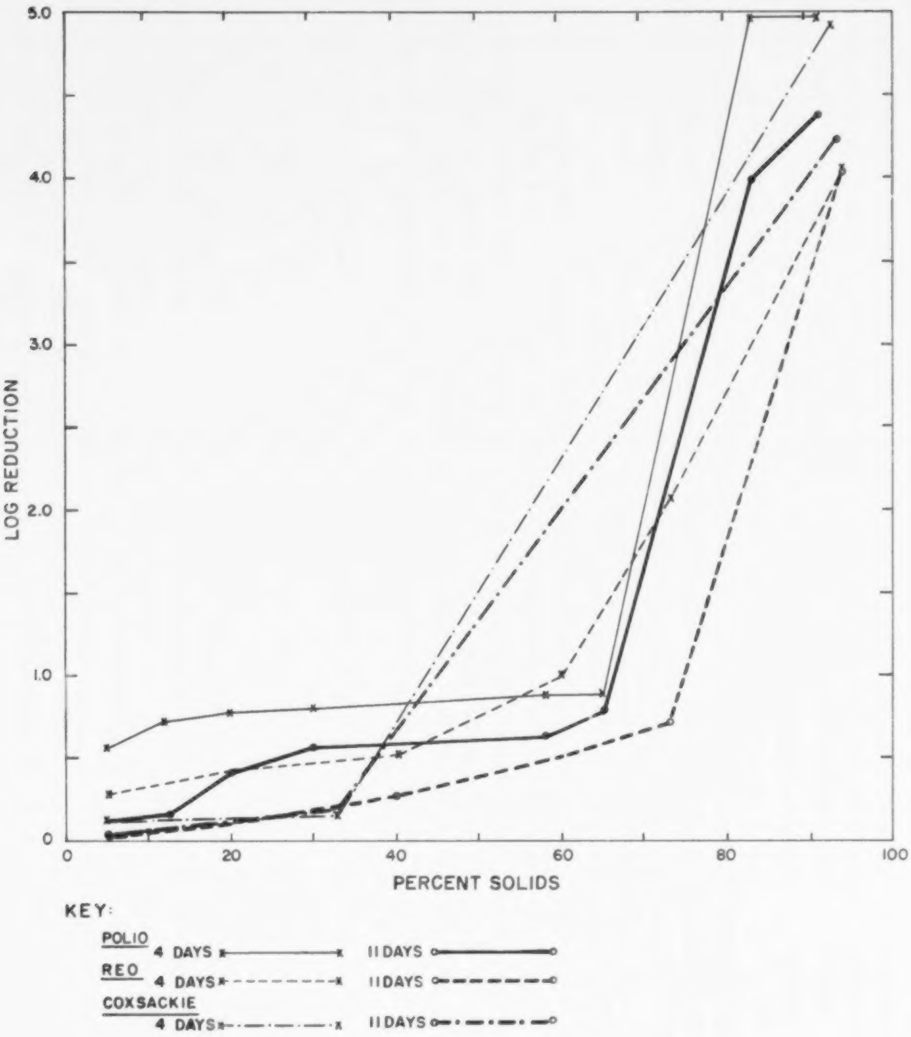

FIGURE 27. REDUCTION OF SEEDED VIRUS VS. SOLIDS CONCENTRATION.*

*WARD AND ASHLEY (1977 b)

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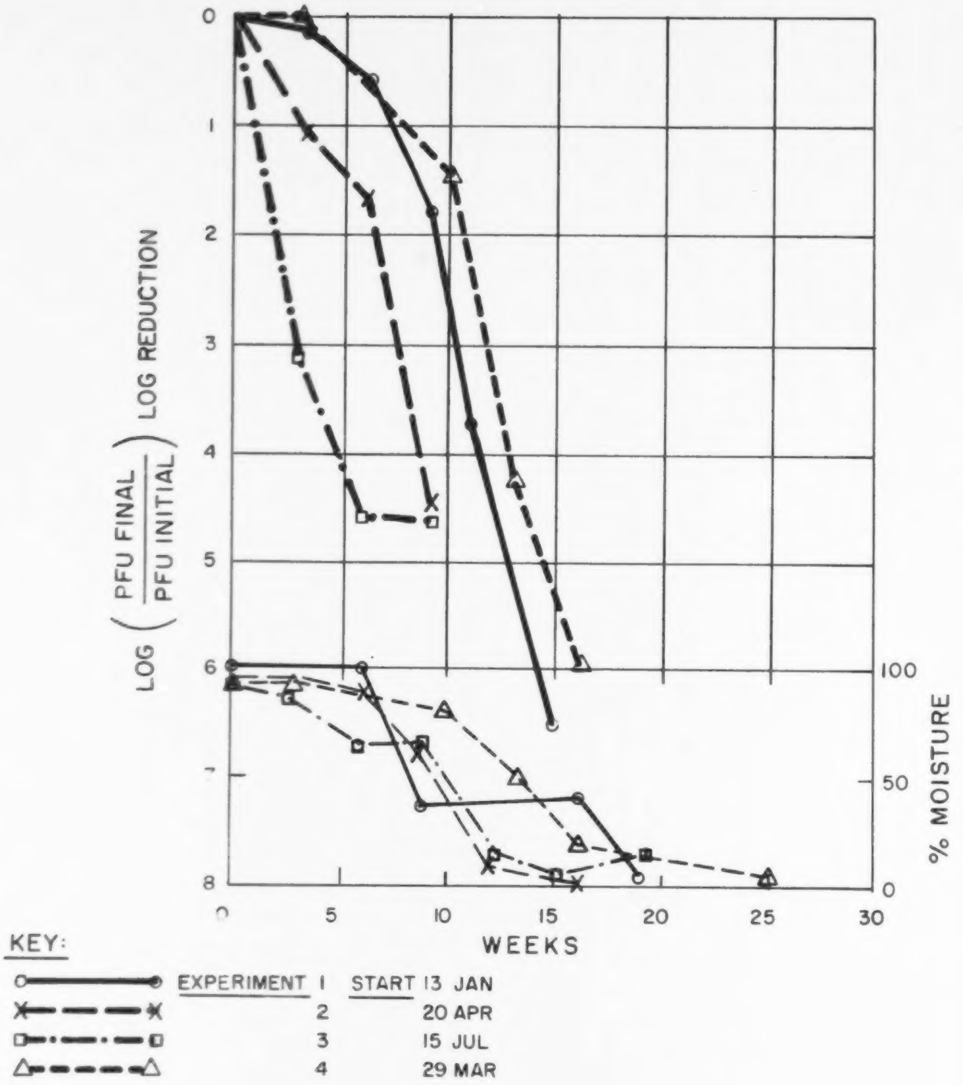

FIGURE 28. REDUCTION OF f_2 BACTERIOPHAGE IN SLUDGE DRYING BEDS.*

* BAXTER et al. (1980), POINTS ARE AVERAGES OF THREE SAMPLES COLLECTED

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In the only report of levels of virus from a full-scale sand drying bed, Wellings *et al.* (1976) reported a concentration of 24 PFU of echovirus type 7 per 250 g of sludge (0.1 PFU/g). The sludge sample collected had been drying for up to 13 days at a full-scale drying bed located in Florida. No data on virus levels before drying were reported in this study.

Parasites. The effects of sandbed dewatering *Ascaris lumbricoides* ova inoculated into anaerobically digested sludge was studied by Cram (1943) on a laboratory scale (Cincinnati, Ohio). No quantitative density data were presented for these experiments; it was reported, however, that viable ova were found after 18 days of indoor drying or 70 days of outdoor drying in uncovered beds. Moisture levels below 10 percent and temperatures of up to 46°C occurred during the experimental period. At moisture levels of less than 3-4 percent, only disintegrated ova were found.

In the same experiment (Cram, 1943), ova of the dog hookworm, *Ancylostoma caninum*, survived and hatched after 62 days of drying during which time solids levels reached 91.1 percent.

Jones *et al.* (1947) and Newton *et al.* (1948) found that with similar moisture levels over a 23-32 day period, time, rather than moisture content, affected the survival of the viable miracidia (ciliated first larvae) of the blood fluke, *Schistosoma japonicum*. In these studies *Schistosoma* ova were seeded into anaerobically digested sludge and dried at temperatures ranging from 16-24°C to 29-32°C for up to 32 days. Viable *Schistosoma* ova were destroyed within 3 weeks at the lower temperatures and within 9 days at the higher. Solids ranged from 3 percent to 91 percent in these experiments.

The studies by Reimers *et al.* (1980) provide information on reduction by digestion and sludge drying combined. Data are not given on the separate processes, however. In these studies on WWTPs in the southern U.S., inactivation was obtained at higher moisture contents than in the studies by Cram (1943). Reimers *et al.* (1980) found that densities of viable and nonviable *Ascaris* ova decreased when the moisture content of the sludge dropped to less than 60 percent.

Using regression analysis, the correlation between *Ascaris* survival and moisture content was calculated by Reimers *et al.* and was found to be statistically significant, while that for *Toxocara* was not. This difference was attributed by the authors to variations due to sampling techniques. From these analyses, Reimers and coworkers graphically estimated that 100 percent destruction of viable *Ascaris* ova would occur at approximately 23-percent moisture, while viable *Toxocara* destruction would occur at 35-percent moisture. Actual field data showed that, dependent on season, *Ascaris* and *Toxocara* inactivation occurs at 5-percent moisture content in the fall, 7-percent in the winter, 8-percent in the spring, and 15-percent in the summer. Partly because of this variation, the authors judged that desiccation was not the only mechanism involved in reducing parasite numbers. Temperature, oxygen content, solar radiation, and time might affect the survival rates.

Baxter *et al.* (1980) noted that drying to below 10-percent moisture inactivated all but a small percentage of ova of *Ascaris* held in earthern basins. At 7.7-percent moisture content, less than 4 percent of the ova were infective. The infective ova represented about 0.16 percent of the original viable ova. Drying to 5-percent moisture or less essentially completed the inactivation.

In these studies, inactivation occurred at somewhat higher levels of moisture than recorded by Reimer *et al*. By the time that the sludge had reached 57.9-percent moisture, the counts of viable ova had decreased by 65 percent.

Bond (1958) examined dried sludge from the Tampa Municipal Sewage Disposal Plant, Florida, for levels of *Ascaris lumbricoides* ova. Digested sludge at this plant is distributed on drying beds for up to 21 days. Solids content of the samples examined ranged from 43.7 to 65.8 percent. *Ascaris* ova ranged from mean values of less than 5 to 520 ova/GDW, of which 10 to 50 percent were viable.

In comparison to data collected in Florida, ova at the sewage treatment works in Johannesburg, South Africa, ranged from 1,538 to 4,564 viable ova/GDW in sludge that had been dried for 1-42 days (Keller and Hide, 1951). Maximum solids content of these samples was 47.75 percent. Viable ova were discovered in a sample dried for an indeterminate length of time to a solids content of 95.6 percent. No conclusions were made as to the relationship of solids content to viability; however, the authors concluded that, based on the irregularity of numbers of viable ova recovered versus length of drying time, there was no direct correlation between duration of drying time and relative viability of ova recovered (Keller and Hide, 1951).

Discussion

The limited data base available permits only general conclusions as to the effectiveness of drying processes in reducing the levels of indicator and pathogenic organisms and viruses. A definite conclusion may be drawn regarding the survival of the parasite *Ascaris lumbricoides*. Studies reviewed indicate the essentially complete inactivation at a solids level of greater than 95 percent (Keller and Hide, 1951; Cram, 1943). Ova of *Schistosoma japonicum* were found to be responsive to the length of dewatering on the basis of two studies (Jones *et al.*, 1947; Newton *et al.*, 1948).

Total coliforms in anaerobically digested sludge at a depth of 15 cm can be reduced by greater than 1 log within 28 days (Fuller and Litsky, 1950), at temperatures greater than 22°C. *Salmonella* was reduced 6 logs within 4 days of drying (Brandon and Neuhauser, 1977) at temperatures from 15-38°C and a sludge depth of 5 cm. Coliform bacteria and *Salmonella* are capable of regrowth, however (Baxter *et al.*, 1980).

The survival of seeded enteroviruses examined in laboratory-scale studies for a maximum of 11 days was shown to be related to solids content (Ward and Ashley, 1977b; Nath and Johnson, 1979). Based on Ward and Ashley's study (1977b), to achieve a 1-log (90-percent) reduction, the following solids level should be obtained:

Poliovirus	65-70% solids
Coxsackievirus	35-45% solids
Reovirus	45-74% solids

Normal operating procedures are to remove dried sludge at approximately 40-percent solids. It can be seen that, according to the work of Ward and Ashley (1977b), this is insufficient to guarantee at least a 1-log (90 percent reduction) in enteroviruses.

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SLUDGE STORAGE/LAGOONING

Introduction

Description of Process. Sludge lagoons are commonly used either to store digested sludge before further treatment or land application, or to provide a means of permanent disposal for the sludge. The lagoons are not suitable for storage of untreated or limed sludges because of their odor and nuisance potential. Typically sludge can be stored in earth or concrete-lined lagoons at depths of 60 to 600 cm for periods ranging from several months to years.

This section will address anaerobic and facultative lagooning. In anaerobic storage, no surface mixing is performed. In facultative lagooning, an aerobic layer is maintained by providing mixing of the surface layer. In both processes, the sludge solids settle to the bottom, where their anaerobic decomposition continues. The supernate is returned to the plant for treatment.

Aerobic storage can also be performed in which the entire depth of the lagoon is aerated. This process is essentially a continuation of aerobic digestion and will not be considered in this section.

Operating Parameters. The performance of lagoons is affected by climate. Both precipitation and low temperatures will inhibit dewatering and rate of volatile solids reduction. Valid analysis of the effects of temperature and length of storage on pathogen reduction can, therefore, be made only by comparison of results within a given season or climatic regime.

Data Base Available. In only one laboratory-scale study, conducted by Stern and Farrell (1977), have the effects of time and temperature been related specifically to survival of indicator and pathogenic bacteria and viruses. Other investigators (Hinsley, 1974; Schatzle, 1969; Subrahmanyan, 1975) have simply examined effects of detention time without controlling temperature.

Review of data reported on samples collected at full-scale treatment plants is limited by lack of documentation on detention times or ambient temperatures (Lue-Hing et al., 1977; Sacramento Area Consultants (SAC), 1979). Accordingly, results from such studies can be cited here, but not analyzed in a meaningful way.

The following is a brief synopsis of the literature considered in this section:

- Arther and Fitzgerald (1977)--Examined for parasite ova samples of anaerobically-digested sludge from the Fulton County storage lagoons, Metropolitan Sanitary District of Greater Chicago.
- Bitton et al. (1980)--A mixture of aerobically and anaerobically digested (2:1) sludge is lagooned at the Agricultural Research Center, Jay, Florida prior to land application. The authors moni-

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tored the survival of enterovirus both in continuously loaded lagoons and in lagoons after addition of digested sludge had been suspended for 6 months.

- Hinesly (1974)--Enumerated fecal coliform bacteria in a sample of anaerobically-digested sludge stored in laboratory for 30 days.

- Lue-Hing et al. (1977)--Anaerobically-digested sludge is barged from the West-Southwest WWTP, Metropolitan Sanitary District of Greater Chicago, Illinois, to Fulton County. The sludge is anaerobically lagooned at that site for approximately 1-2 years prior to land application. Analyses were performed for total coliform, fecal coliform and fecal streptococcus bacteria; Staphylococcus aureus; Pseudomonas aeruginosa; Salmonella sp.; and enteric viruses. Additionally, a pilot-scale lagooon was established to determine concentrations of virus present in sludge and to monitor the die-off of viruses in lagooned sludge.

- Sacramento Area Consultants (1979)--The Sacramento Regional County Sanitation District operates facultative lagoons (approximately 2.4 hectares area, 3.6 m depth) for the storage of anaerobically-digested sludge prior to dedicated land disposal. Surface mixers maintain the aerobic surface layer of 30-90 cm. Average detention time is 2-4 yr. Feeding is halted 30-60 days prior to harvesting. Quarterly analyses for fecal coliform and fecal streptococcus bacteria, Salmonella, Shigella, and parasitic ova and cysts, were conducted over a 2-yr period.

- Sattar and Westwood (1979)--Examined anaerobically-digested sludge from a storage lagoon at the Green Creek Pollution Control Center, Ottawa, Canada. Samples were collected over an 11-month period after the loading of fresh sludge to the lagoon was halted. Samples that were positive for viruses were reported.

- Schatzle (1969)--Suspended the ova of Ascaris lumbricoides var suum in waste activated sludge, which was then stored under anaerobic conditions. Viability was determined after 7 months detention.

- Stern and Farrell (1977)--Examined the effects of long-term (6-months) storage of anaerobically-digested sludge at 4°C and 20°C on a laboratory scale. Levels of total and fecal coliform and fecal streptococcus bacteria, Salmonella, and enteroviruses were enumerated. Additionally, qualitative examination of samples for Ascaris lumbricoides were performed.

- Subrahmanyan (1975)--Seeded sludge (type not specified) with a variety of echoviruses, coxsackieviruses and polioviruses. Samples were held at room temperature for up to 12 weeks.

Results

Indicator Bacteria. The significance of temperature-of-storage on the survival of indicator bacteria is evidenced in Table 73 (Stern and Farrell, 1977). At 20°C, reductions of greater than 1 log in densities of total and fecal coliform bacteria and fecal streptococcus bacteria were achieved within 1 month. At 4°C, a 1-log reduction of total coliform bacteria was not achieved until after about 3 months of storage. Fecal streptococcus bacteria were not reduced appreciably, even after 6 months at 4°C.

Hinesly (1974) obtained similar reductions of fecal coliform bacteria to those reported by Stern and Farrell (1977), but did not specify the temperature of storage (Table 74).

Greater than 1-log reductions of total and fecal coliform bacteria are achieved by lagooning anaerobically-digested sludge for an approximate detention time of 1-2 yr (Table 75) and 2-4 yr (Table 76).

Poorer reductions of fecal streptococcus bacteria reported by the Sacramento Area Consultants (SAC)--averaging 0.96 logs over a 2-yr period--are in agreement with those reported by Stern and Farrell (1977) for laboratory-scale storage at lower temperatures.

Density levels of fecal coliform and fecal streptococcus bacteria have been compared in seven continuously loaded lagoons to those in another basin (SSB-3) where loading was halted for a 5-month period, beginning in May 1977. Figure 29 presents a comparison of the results. Inexplicable increases in the density of fecal streptococcus bacteria occurred for a time after the cessation of loading. After 5 months of storage (without loading), however, lower levels of fecal streptococcus and fecal coliform bacteria resulted, as compared to the levels in basins that had received continuous loading. The researchers observed a difference of 0.72 logs and 0.86 logs compared to continuously fed lagoons for fecal streptococcus and fecal coliform bacteria, respectively.

Pathogenic Bacteria. At a temperature of 4°C, a 1-log reduction of *Salmonella* sp. was not achieved until after 6 months of anaerobic storage (Stern and Farrell, 1977). At 20°C, a greater than 1-log reduction occurred within 1 month (Table 77).

The low levels of *Salmonella* sp. reported in anaerobically-digested sludge at full-scale treatment plants prevent consideration of the reductions that may occur during subsequent lagooning. There is no significant difference between the very low initial densities of *Salmonella* in digested sludge and the limits of detection that were encountered in attempts to enumerate these bacteria after lagooning (Tables 78 and 79).

Log reductions of 2.08 and 0.94 in densities of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively, were reported by Lue-Hing *et al.* (1977) after barging and lagooning of digested sludge (Table 78).

TABLE 73. DENSITIES OF INDICATOR BACTERIA DURING ANAEROBIC STORAGE OF ANAEROBICALLY-DIGESTED SLUDGE ON A LABORATORY SCALE AT 4° AND 20°C*

	Total Coliform		Fecal Coliform		Fecal Streptococcus	
	4°	20°	4°	20°	4°	20°
Initial density	2.6×10^{8}	2.6×10^{8}	8.5×10^{7}	8.5×10^{7}	3.1×10^{6}	3.1×10^{6}
1 month, density	4.8×10^{7}	1.8×10^{5}	1.4×10^{6}	5.0×10^{4}	2.5×10^{6}	1.0×10^{5}
1 month, log reduction	0.73	3.16	1.78	3.23	0.09	1.49
2 months, density	3.0×10^{7}	3.0×10^{4}	3.5×10^{5}	5.5×10^{3}	1.5×10^{6}	2.5×10^{4}
2 months, log reduction	0.94	3.94	2.39	4.19	0.32	2.09
4 months, density	3.7×10^{6}	6.0×10^{3}	1.5×10^{5}	6.0×10^{3}	1.3×10^{6}	<3
4 months, log reduction	1.85	4.64	2.75	4.15	0.38	>6.01
6 months, density	1.0×10^{3}	$<1.0\times10^{3}$	1.0×10^{3}	$<1\times10^{3}$	5.1×10^{6}	<3
6 months, log reduction	5.41	>5.41	4.93	>4.93	-0.22	>6.01

*Stern and Farrell (1977). Densities in no./100 ml.

TABLE 74. REDUCTION OF FECAL COLIFORM BACTERIA DURING STORAGE OF ANAEROBIC SLUDGE FOR 30 DAYS ON A LABORATORY SCALE*

Initial	Final	Log Reduction
4×10^{6}	2×10^{4}	2.3

*Hinesly (1974). Count/100 ml. No temperature or condition of storage specified.

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TABLE 75. DENSITY LEVELS OF INDICATOR BACTERIA BEFORE AND AFTER ANAEROBIC LAGOON STORAGE*

Organism	Digester Draw[a]			Barged Sludge[b]			Lagooned Sludge[c]			Log Reduction[d]
	Mean	N	Range	Mean	N	Range	Mean	N	Range	
Total coliform bacteria	1.3×10^6	NS[e]	NS	2.5×10^5	NS	NS	1.2×10^5	NS	NS	1.03
Fecal coliform bacteria	1.4×10^5	NS	NS	1.2×10^4	NS	NS	9.8×10^3	NS	NS	1.15
Fecal strepto-coccus bacteria	1.2×10^5	NS	NS	3.4×10^4	NS	NS	1.9×10^3	NS	NS	1.80

*Count/100 ml

[a] High-rate anaerobic digestion at 35°C for 14-15 days
[b] Digester draw plus an unspecified amount of stored solids barged from the West Southwest Plant, MSDGC to Fulton County
[c] Sludge lagooned prior to land application
[d] Reduction calculated from initial levels in digester draw
[e] Not specified by author

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TABLE 76. DENSITY LEVELS OF FECAL COLIFORM AND FECAL STREPTOCOCCUS BACTERIA BEFORE AND AFTER FACULTATIVE LAGOON STORAGE*

	Fecal Coliform				Fecal Streptococcus				
Sludge Type	Mean	N	Range	Log Reduction	Mean	N	Range	Log Reduction	
Digested[a]	$1.1x10^{6}$	4	NS [d]	-	$1.2x10^{6}$	4	NS	-	1976
Lagoons[b]	7.9x103	24	NS	2.14	$9.6x10^{4}$	24	NS	1.10	
Harvested[c]	$1.7x10^{3}$	2	NS	2.81	$3.7x10^{5}$	2	NS	0.51	
Digested[a]	$1.3x10^{4}$	4	NS	-	$6.9x10^{4}$	4	NS	-	1977
Lagoons[b]	$8.9x10^{2}$	32	NS	1.16	$1.3x10^{4}$	32	NS	0.72	
Harvested[c]	$4.7x10^{1}$	5	NS	2.44	$2.7x10^{3}$	5	NS	1.41	
Average Log Reduction of Harvested Sludge				2.63				0.96	

*Sacramento Area Consultants (1979). Count/GDW.

[a] Composite samples from middle and bottom of high-rate anaerobic digester
[b] Lagoons are approximately 2.4 ha in area and 360 cm in depth. Geometric mean of 8 composite samples collected on each of 4 sampling dates.
[c] Harvested sludge is dredged from the bottom of storage basins. Average detention 2-4 years. Sludge feeding is halted 30-60 days prior to harvesting.
[d] NS= Not specified by author.

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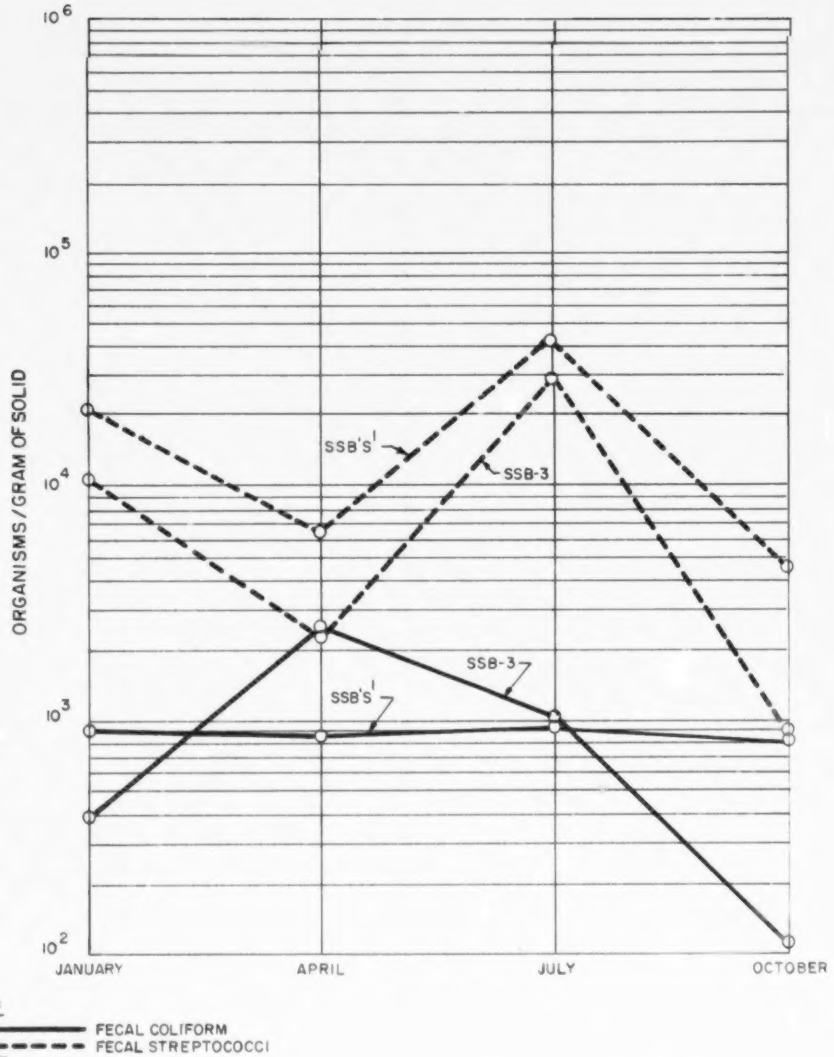

NOTE:
1 - GEOMETRIC MEAN, NOT INCLUDING SSB-3

FIGURE 29. COMPARISON OF FECAL COLIFORM AND FECAL STREPTOCOCCI IN SSB-3 (LOADING HALTED) AND THE COMBINED REMAINING SEVEN SSB'S (CONTINUOUS LOADING) 1977.*

* SACRAMENTO AREA CONSULTANTS (1979)

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TABLE 77. REDUCTION OF SALMONELLA SP. DURING ANAEROBIC STORAGE OF DIGESTED SLUDGE ON A LABORATORY SCALE*

	4°	20°
Initial density	2.4×10^2	2.4×10^2
1 month, density	2.4×10^2	3.6×10^0
1 month, log reduction	0	1.82
2 months, density	2.7×10^2	<3
2 months, log reduction	-	>1.90
4 months, density	1.3×10^2	<3
4 months, log reduction	0.27	>1.90
6 months, density	<3	<3
6 months, log reduction	>1.90	>1.90

*Stern and Farrell (1977). No./100 ml

TABLE 78. DENSITY LEVELS OF SALMONELLA SP, PSEUDOMONAS AERUGINOSA, AND STAPHYLOCOCCUS AUREUS BEFORE AND AFTER LAGOON STORAGE*

Organism	Digester Draw[a]			Barged Sludge[b]			Lagooned Sludge[c]			Log Reduction[d]
	Mean	N	Range	Mean	N	Range	Mean	N	Range	
Salmonella	1.6×10^0	NS[e]	NS	2.0×10^{-1}	NS	NS	ND[f]	NS	NS	-
Pseudomonas aeruginosa	7.1×10^5	NS	NS	3.2×10^5	NS	NS	5.9×10^3	NS	NS	2.08
Staphylococcus aureus	2.5×10^4	NS	NS	1.4×10^5	NS	NS	2.9×10^3	NS	NS	0.94

*Lue-Hing et al. (1977). Count/100 ml. Counts are geometric mean of analyses performed on samples collected quarterly (1975) and monthly (1976)

[a] High rate anaerobic digestion for 14-15 days at 35°C

[b] Digester draw plus an unspecified amount of stored solids which are barged from the West-Southwest WTP, MSDGC to Fulton County

[c] Sludge lagooned prior to land application

[d] Reduction calculated from initial levels in digester draw

[e] Not specified by author

[f] Not detected. Analytical detection limits not specified by author.

TABLE 79. DENSITY LEVELS OF SALMONELLA AND SHIGELLA SPP. (ENUMERATED SIMULTANEOUSLY) BEFORE AND AFTER LAGOON STORAGE*

	1976				1977[d]			
Sludge type	Mean	N	Range	Log Reduction[g]	Mean	N	Range	Log Reduction
Digested[a]	ND[e]	4	NS[f]	-	$3.8x10^{-1}$	4	NS	-
Storage basin[b]	ND	24	NS	-	$5.6x10^{-1}$	32	NS	-
Harvested[c]	ND	2	NS	-	$2.4x10^{-1}$	5	NS	-

*Sacramento Area Consultants (1979). Densities in MPN/GDW

[a] Composite samples from middle and bottom of digester

[b] Storage basins are approximately 2.4 ha in area and 360 cm in depth. Average detention is 2-4 years. Geometric mean of 8 composite samples collected on each of 4 sampling dates.

[c] Harvested sludge is dredged from the bottom of storage basins. Sludge feeding is halted 30-60 days prior to harvesting

[d] Reported geometric mean of one half the detection limit

[e] ND = Not detected

[f] NS = Not specified by author

[g] Data is not amenable to log reduction calculations for the reason cited in (d) above

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Viruses. Subrahmanyan (1975) compared the survival of different serotypes of viruses seeded into raw sludge and stored at 22-25°C (Figure 30). He reported that coxsackievirus B2 persisted for 2 weeks, while B5 was still present after 12 weeks; and that echovirus 6 was not detected after 10 weeks, while echovirus 9 was detected even after 12 weeks.

Subrahmanyan (1975) also compared survival of virulent (wild) poliovirus strains to those of vaccine strains and found no consistent differences. Poliovirus type 1 vaccine was less persistent than the virulent virus, but type 3 virulent virus was more persistent than the vaccine strain.

The reductions in enterovirus observed when raw sludge is stored cannot be validly compared with reductions that may occur in digested sludge. Factors that have been shown to be virucidal in digested sludge--such as ammonia (Ward and Ashley, 1977; Fenters et al., 1979)--would be present at lower concentrations in raw sludge.

Indigenous enterovirus were reported by Stern and Farrell (1977) to survive far longer at 4°C than at 20°C. As shown in Table 80, a 0.48-log reduction occurred after 6 months at 4°C; at 20°C, a greater than 1-log reduction occurred within 2 months.

No viruses were detected by Lue-Hing et al. (1977) in anaerobically-digested sludge samples that were collected over a 2-yr period. Samples were collected from the digester draw and from anaerobic lagoons.

Sattar and Westwood (1979) conducted a 15-month study of viruses in raw, anaerobically digested, and lagooned sludge in Ottawa, Canada. While quantitative analyses were not performed, reovirus was detected after 8 months of storage (in 1 of 3 samples). No viruses were detected in single samples collected after 13 and 14 months' retention (Table 81).

Bitton et al. (1980) showed that lagooning of a mixture of aerobically and anaerobically (1:2 mixture) digested sludge, in the warm climate of Florida, may be an efficient means of reducing viral numbers (Table 82). Enteroviruses were readily recovered from lagoons at concentrations of 75 $TCID_{50}$/gram dry weight (GDW) while digested sludge was being added to the lagoon. Two months after addition of sludge to the lagoon had been suspended, and for the next 6 months (June-November), no viruses were detected in samples of lagooned sludge. They were again detected when the addition of digested sludge to the lagoon was resumed (Figure 31). Enteroviruses were also found in the supernatant water when sludge was being added to the lagoon and were not found when sludge addition was suspended (Bitton et al., 1980).

Parasites. Qualitative results indicate that viable *Ascaris lumbricoides* in digested sludge can survive for greater than 6 months during anaerobic storage at 4°C and 20°C (Stern and Farrell, 1977). Quantitative analyses performed by Veerannan (1977) found that 50 percent of seeded ova of *A. lumbricoides* were still viable after 1 yr, 12 percent after 2 yr, and practically none after 3 yr of laboratory-scale storage.

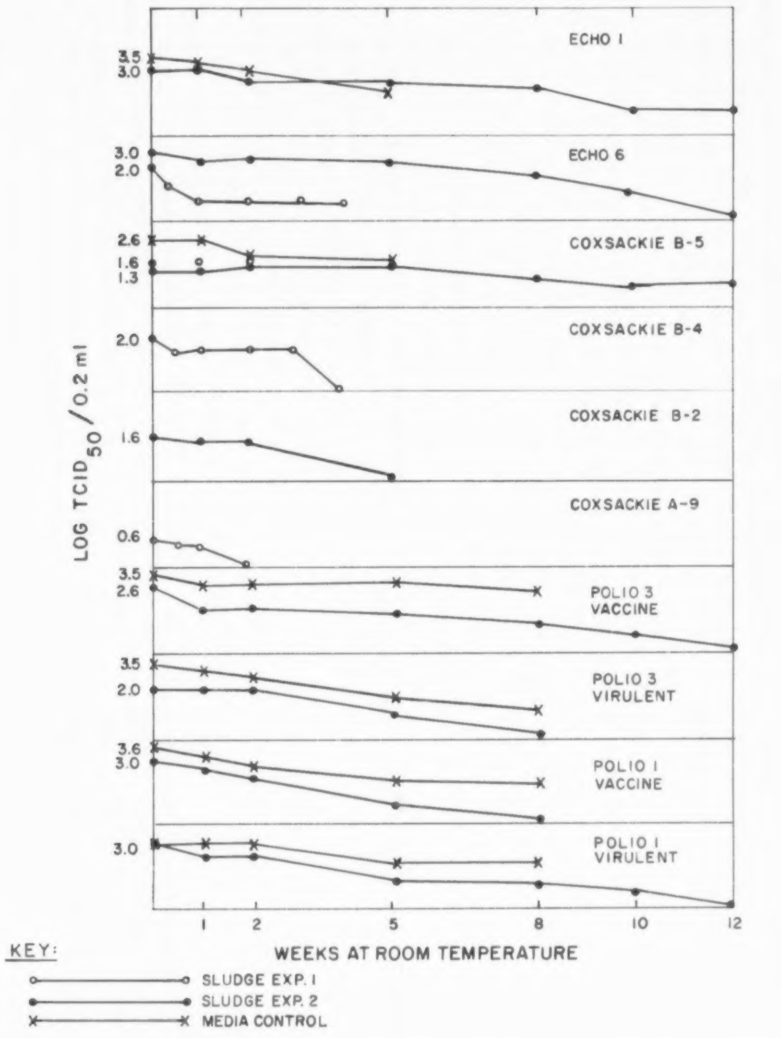

FIGURE 30. REDUCTION OF SEEDED ENTEROVIRUS STORED IN RAW SLUDGE AT ROOM TEMPERATURE.*

* SUBRAHMANYAN (1975)

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TABLE 80. REDUCTION OF INDIGENOUS ENTEROVIRUSES DURING STORAGE OF ANAEROBICALLY DIGESTED SLUDGE ON A LABORATORY SCALE*

Time (months)	4°C		20°C	
	Enterovirus	Log Reduction	Enterovirus	Log Reduction
0	84	-	124	
1	92	-0.04	24	0.71
2	108	-0.11	<4	>1.49
4	52	0.21	<4	>1.49
6	28	0.48	<4	>1.49

*Stern and Farrell (1977). Densities in PFU/15 g extract. 15 g of sample were extracted with 40 ml of buffered beef extract. 10 ml of this extract was assayed for Plaque Forming Units.

TABLE 81. PERCENTAGE OF SAMPLES THAT WERE POSITIVE FOR INDIGENOUS ENTEROVIRUS DURING LAGOON STORAGE OF ANAEROBICALLY-DIGESTED SLUDGE*

Time (month)	% of Samples That Were Positive	No. Samples
1	100	2
2	33	6
3	66	3
4	40	5
7	50	4
8	33	3
9-14	0	4

*Sattar and Westwood (1979)

TABLE 82. REDUCTION OF INDIGENOUS ENTEROVIRUS DURING LAGOON STORAGE OF SLUDGE*

Time (months)	Enterovirus	Log Reduction
0	75	-
1	60	0.10
3	<0.1	>2.88
7	<0.1	>2.88

*Bitton et al. (1980). Densities in $TCID_{50}$/GDW. Feed sludge: mixture of 1/3 aerobically-digested sludge to 2/3 anaerobically-digested sludge.

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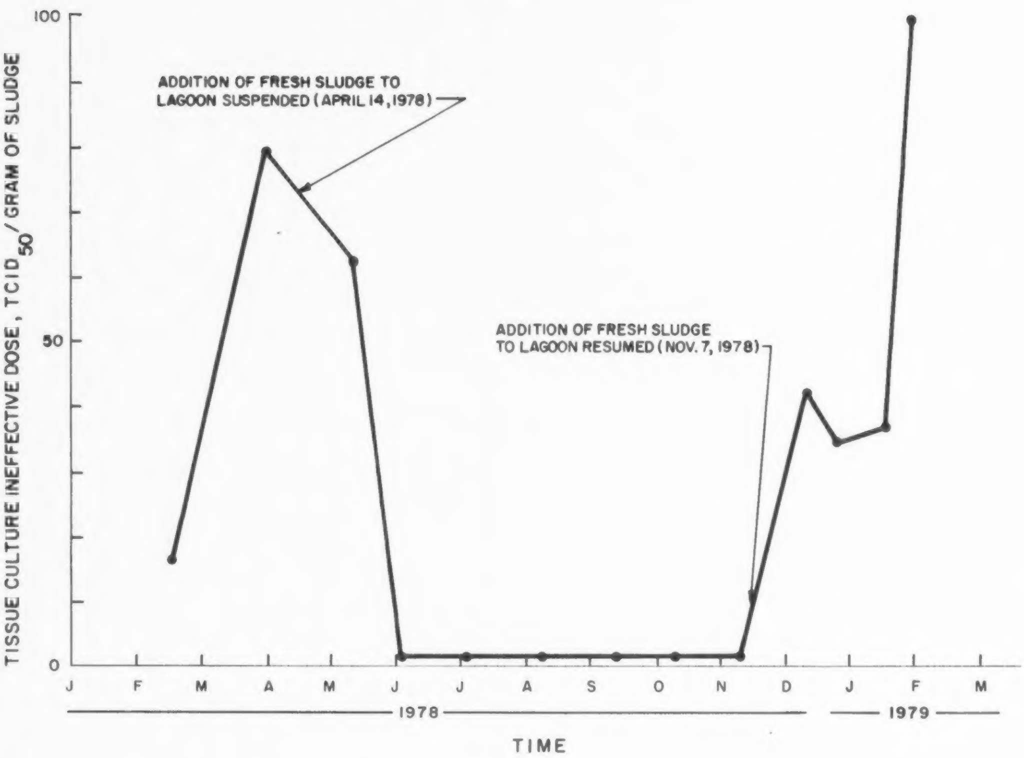

FIGURE 31. LEVELS OF ENTEROVIRUS IN LAGOONED, COMBINED SLUDGE.*

* BITTON et al. (1980)

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Levels of viable ova of *Ascaris lumbricoides* var *suum* were not reduced after 3 months storage in contact with sludge from an oxidation ditch at temperatures from 0 to 20°C. After 7 months, a 10-percent reduction in viable ova was found (Schatzle, 1969).

As part of the microbiological studies conducted by the SAC (1979) previously described, sludge samples were examined qualitatively for the presence of parasitic helminths and protozoans (Table 83).

Three positive samples were collected in 1976, and two in 1977. *Toxacara canis* (the dog ascarid) was isolated at a density of less than 50 ova/GDW in both of the positive 1977 samples. The authors note that the recoveries they report in digested sludge are low compared to other authors (La Conde, 1978) and cite the small number of samples collected/site and methodology utilized as partial explanation for the low density of parasites reported. Arther and Fitzgerald (1977) reported that viable nematode ova were found in digested sludge stored in lagoons for up to 5 yr.

Discussion

Two parameters--temperature, and length of storage time--have been studied with regard to effect on the survival of indicators, pathogens and parasites in sludge stored in anaerobic or facultative lagoons. At lower temperatures, a longer detention time is required to achieve significant (i.e., greater than 1-log) reductions.

On the basis of a very limited and essentially laboratory-scale data base, the following trends are indicated. At temperatures of 20°C or greater, the minimum storage time required to achieve a 1-log reduction is 1 month for bacteria, 2 months for viruses, and greater than 6 months for parasitic ova. At temperatures of less than 20°C, more than 6 months storage is required to reduce pathogenic bacteria by 1 log, more than 8 months for viruses, and at least 3 yr for the reduction of parasitic ova.

Lagooning of digested sludge for 1 to 4 yr at two full-scale treatment plants resulted in *Salmonella* densities of 1 organism/GDW or 1 organism/100 ml. The low levels of this pathogen prevent any comparisons of its reduction to that of the traditional indicator bacteria.

It should be noted, however, that the fecal streptococcus bacteria appear to be the most resistant bacteria of the indicator groups, and thus would be the most conservative indicator of pathogen reduction.

TABLE 83. PERCENTAGE OF SAMPLES THAT WERE POSITIVE FOR PARASITES BEFORE AND AFTER SLUDGE STORAGE*

	1976		1977	
Sludge Type	No. Samples Examined	No. Positive	No. Samples Examined	No. Positive
Digested	4	0	4	1
Storage basins	24	2	32	1
Harvested	3	1	6	0

*Sacramento Area Consultants (1979)

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SLUDGE CONDITIONING/MECHANICAL DEWATERING

Introduction

Description of Processes. At many wastewater-treatment plants, sludges are dewatered by mechanical means prior to further processing or disposal. Dewatering--whether by vacuum filter, chamber filter press, belt filter press or centrifuge--typically yields a sludge cake with solids concentrations in the range of 15 to 40 percent. In common practice, chemicals are applied to the sludge prior to mechanical dewatering in order to improve its dewatering characteristics.

Mechanical dewatering in itself has little effect on pathogens in the sludge (LeClerc *et al.*, 1970). Moisture remaining in the sludge cake will support microbial populations, and bacterial food sources remain unaffected. It is the application of chemical conditioners--substances intended only to improve dewaterability--that can reduce densities of indicator or pathogenic organisms in the sludge-dewatering process. The sludge conditioners that are commonly used include lime (CaO), ferric chloride ($FeCl_3$), ferrous sulfate ($FeSO_4$), and polyelectrolytes or polymers. Typical dewatering systems are operated as follows:

Dewatering Unit	Conditioning Chemical	Application
Belt filter press Centrifuge	Polymer	Added to conditioning drum for detention time of about one minute, or injected directly into sludge pipeline.
Vacuum filter	Ferric chloride Ferric chloride and lime Polymer Ferrous sulfate and lime (less common)	Added to conditioning drum, detention time of up to 15 minutes in filter vat prior to dewatering.
Chamber filter press	Ferric chloride and lime Polymer (rare)	Added to rapid-mix and conditioning tanks, total detention time of 45 minutes or more prior to dewatering.

Operating Parameters. Significant kills of indicator and pathogenic organisms evolve incidentally from the use of conditioning chemicals, which are applied to sludge in quantities that will economically improve sludge dewaterability. As with lime stabilization of sludge, the variables that affect the degree of microorganism kill are pH (which varies with the chemical dose applied and the type of sludge being conditioned) and detention time. It should be noted, however, that the conditioning step is designed to optimize dewaterability and not to effect kill of pathogens. Sludge pH levels are not routinely monitored in the conditioning operation. Conditioning tanks are sized not to afford maximum detention time, but simply to provide a relatively brief, gentle mixing of sludge with conditioning chemicals. Use of ferric chloride in conjunction with lime, as is routinely practiced, counteracts the high pH that would be achieved through use of lime alone; this reduces the effectiveness of lime as a bactericidal or virucidal agent.

Data Base Available. Only a handful of studies have been conducted on reductions of microorganisms occurring as a result of conditioning and dewatering sludge. Three types of systems have been tested:

a. Full-scale dewatering systems (normal operation)--Kampelmacher and van Noorle Jansen (1972) studied levels of aerobic microorganisms, Enterobacteriaceae (including coliform bacteria, *Salmonella*, and related organisms), and gram positive bacilli and cocci bacteria in sludges conditioned by a lime/ferric-chloride mixture and dewatered on vacuum filter. Shook *et al*. (1962) examined densities of total coliform bacteria in raw and lime-conditioned sludge prior to dewatering.

b. Full-scale dewatering systems (variable chemical doses and pH values)--Doyle (1967) tested for the presence of the pathogen *Salmonella typhosa* in raw sludge and in sludge conditioned by lime in a full-scale system to pH levels ranging from 10.5 to 12.0.

c. Batch conditioning and dewatering (pilot and laboratory scale)--Trubnick and Mueller (1958) conducted a filtration study in which survivals of coliform bacteria were reported for pH values achieved by use of varying doses of ferric chloride with lime. Farrell *et al*. (1974) conducted bench-scale tests in which lime was added to liquid sludge and the sludge dewatered by a vacuum-filter leaf; effects of lime dose, pH, contact time prior to filtering, and storage of liquid and cake sludges were studied as they affected survivals of total aerobic organisms, fecal coliform and fecal streptococcus bacteria, *Salmonella* and *Pseudomonas aeruginosa*, hookworms, amoebic cysts and *Ascaris* ova.

In much of the available literature on pathogen kills in sludge-dewatering processes, data and descriptions of test procedures were incomplete. In order to draw useful and valid generalizations, data should include:

- Sludge source
- A list of all upstream processes (including storage)
- Sludge solids concentration
- Chemical dose
- pH
- pH and detention time relationships
- Time from chemical dosage until filtering
- Final solids concentration
- Final pH
- Pathogen and indicator organism determinations on raw sludge, liquid sludge with lime (or other conditioning chemical), and sludge cake

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- o Complete descriptions of equipment and test procedures

Without such a complete data base, it is possible only to outline apparent relationships and areas requiring further study.

Results

Only two investigators have studied the bactericidal effects of conditioning chemicals other than lime. Trubnick and Mueller (1958) tested the effects on coliform bacteria of increasing doses of ferric chloride added to sludge combination with an unstated, constant lime dose; their findings are presented in Table 84. They suggest that ferric chloride decreases the colicidal effects of lime because it decreases the pH that would be achieved through use of lime alone.

LeClerc et al. (1970) counted microorganisms in raw sludges and in sludges conditioned variously prior to dewatering by vacuum filters, filter presses, and centrifuges. In samples conditioned with polymer or in those receiving no conditioning prior to dewatering, no reduction in microbial densities was observed. In samples conditioned with lime, there did occur a reduction in microorganisms.

Although use of ferric chloride alone might lower pH to levels that inhibit bacterial growth (but not viral viability), the literature does not provide data to this effect. Lime is the only chemical conditioner in common use that has been demonstrated to kill indicator and pathogenic organisms. Lime's effectiveness depends on the high pH levels attained, as we have seen in a previous section. The remaining studies on conditioning/dewatering of sludge deal exclusively with the effects of high pH induced by application of lime.

Indicator Organisms. In three studies, lime conditioning has been examined with respect to its effects on indicator organisms.

Shook et al. (1962) compared presumptive coliform-bacteria counts in raw and conditioned sludge prior to dewatering at full-scale operation. The lime dose used was not recorded, but was selected solely to achieve good dewaterability. Results showed that 99 to 99.97 percent of the presumptive coliform-bacteria density was destroyed by addition of lime, representing a two- to four-log reduction from an initial density of 4.5 x 10^6 per 100 ml. The pH level was not reported.

The laboratory-scale data of Farrell et al. (1974) are the most important available on the effectiveness of lime conditioning for sludge stabilization, in that the researchers considered three major variables--pH, detention time, and effect of dewatering. Samples were limed to an initial pH of 11.5, filtered immediately, and held as a cake for tests at 0.5 hr and 24.0 hr. (Other samples were limed and held in a liquid state for up to 24 hr; data on these tests are presented in Tables 58 and 59 describing lime stabilization of sludge.) Results are presented in Table 85.

Kampelmacher and van Noorle Jansen (1972) reported the number of aerobic

TABLE 84

EFFECT OF FERRIC CHLORIDE ON COLIFORM BACTERIA*

Ferric Chloride Dose**	pH	MPN/dry g
0.9	8.3	6.3×10^3
1.8	7.3	2.5×10^4
2.7	7.2	1.0×10^3
4.2	5.6	1.0×10^7

* From Trubnick and Mueller (1958)

** Percent $FeCl_3$: Lime dose, sludge type and characteristics not reported

TABLE 85

DENSITIES OF INDICATOR ORGANISMS IN LABORATORY SYSTEM WITH FILTRATION TWO MINUTES AFTER LIME ADDITION TO pH 11.5*

Indicator	Density (no. per dry gram)		
	Raw	0.5 hr	24.0 hr
Fecal Coliform Bacteria	2.5×10^7	1.3×10^5	5.5×10^5
Fecal Streptococcus Bacteria	1.9×10^6	1.4×10^6	1.5×10^6

* From Farrell _et al_. (1974)

microorganisms, gram positive bacilli and cocci, and Enterobacteriaceae in sludge before after full-scale conditioning with lime and either ferric chloride or ferrous sulfate and dewatering on a vacuum filter. The classes of organisms studied by these researchers all include both indicators and important human pathogens.

Results are presented in Table 86.

Pathogenic Bacteria. Only Farrell et al. (1974) and Doyle (1967) have examined conditioning/dewatering processes with respect to reductions in densities of pathogenic organisms. In the study by Farrell et al., samples were treated by lime to an initial pH of 11.5, filtered immediately, and held as a cake for tests at 0.5 hr and 24.0 hr. Densities of *Salmonella* sp. and *Ps. aeruginosa* in raw and treated sludge are recorded in Table 87.

Doyle studied presence--not density--of *Salmonella typhosa* in sludge after full-scale lime conditioning. All four samples with an initial pH of 8.0 or less showed the presence of *S. typhosa*. Of seven samples with initial pH levels in the range of 10.5 to 12.0, five samples showed the presence of the pathogen. Only one of five samples limed to an initial pH of greater than 12.0 showed the presence of *S. typhosa*.

All nine samples with a pH of 10.0 or less after 24 hr detention contained *S. typhosa*, but none of six samples at pH 10.5 or greater showed its presence.

Viruses. LeClerc et al. (1970) studied inactivation of enteroviruses in the sludge-conditioning/dewatering process. Sludge was dewatered on filter-press or vacuum-filter without use of conditioning chemicals, or was conditioned with lime or polymer prior to dewatering. Results are presented in Table 88.

Parasites. Farrell et al. (1974) conducted a microscopic examination of lime-conditioned sludges in order to determine whether higher parasitic organisms could survive treatment to a pH of 11.5. The researchers found, after 24-hr detention time, that hookworms, amoebic cysts and *Ascaris* ova all survived the lime conditioning.

Discussion

The limitations of the existing data base make possible few generalizations about the effectiveness of conditioning/dewatering processes in reducing levels of indicator and pathogenic microorganisms in sludge. Moreover, the fact that most full-scale installations do not monitor the parameters that affect pathogen kills limits the practical applicability of information obtained in laboratory- and pilot-scale studies.

Dewatering alone appears to have no significant effect on densities of microorganisms in sludge (Table 88). Among the conditioners commonly used to improve sludge dewatering characteristics, polymer does not appear to reduce densities of enteroviruses (Table 88). Ferric chloride, which is

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TABLE 86

DENSITIES OF THREE TYPES OF INDICATOR AND PATHOGENIC ORGANISMS BEFORE AND AFTER LIME CONDITIONING AND DEWATERING*

	Density (no. per dry gram)					
Sludge Type	Aerobic Microorganisms		Enterobacteriaceae		Gram Positive Bacilli and Cocci	
	Range	Mean	Range	Mean	Range	Mean
Primary						
Raw (4% solids)	$6x10^5 - 3x10^7$	$4x10^6$	$5x10^4 - 1x10^7$	$8x10^5$	$7x10^3 - 1x10^6$	$8x10^4$
Treated (3-4% lime and 0.5-2.5% ferric chloride; 25% solids)	$3x10^3 - 3x10^5$	$2x10^4$	$2x10^2 - 2x10^4$	$2x10^3$	$5x10^1 - 8x10^3$	$5x10^2$
Primary Plus Waste Activated Sludge						
Raw (9% solids)	$8x10^7 - 8x10^8$	$3x10^8$	$6x10^7 - 4x10^8$	$2x10^8$	$8x10^5 - 8x10^6$	$3x10^6$
Treated (3-4% lime and 0.5-2.5% ferric chloride; 26% solids)	$1x10^4 - 8x10^5$	$1x10^5$	$3x10^3 - 1x10^5$	$2x10^4$	$2x10^2 - 1x10^5$	$5x10^3$
Primary Plus Waste Activated Sludge						
Raw (8% solids)	$3x10^5 - 3x10^7$	$3x10^6$	$2x10^5 - 3x10^7$	$2x10^6$	$3x10^4 - 1x10^6$	$2x10^5$
Treated (10% lime and 2% ferric chloride; 27-30% solids)	$7x10^3 - 7x10^4$	$2x10^4$	$1x10^2 - 8x10^3$	$1x10^3$	$5x10^1 - 9x10^3$	$7x10^2$

* From data of Kampelmacher and van Noorle Jansen (1972)

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TABLE 87

DENSITIES OF PATHOGENIC ORGANISMS IN LABORATORY SYSTEM WITH FILTRATION TWO MINUTES AFTER LIME ADDITION TO pH 11.5*

Pathogen	Density (no. per dry gram)		
	Raw	0.5 hr	24.0 hr
Salmonella	>700	1.9	1.9
Pseudomonas aeruginosa	>300	0.8	4.2

* From Farrell *et al.* (1974)

TABLE 88

EFFECT OF CONDITIONING/DEWATERING ON ENTEROVIRUSES*

Chemical Dose	Dewatering Device	Percent of Sludge Samples Testing Positive for Enterovirus
None	Filter press	100
0.5% polymer	Filter press	100
10% lime	Filter press	33
10% lime	Vacuum filter	0
15% lime	Vacuum filter	0
20% lime	Vacuum filter	0
25% lime	Vacuum filter	0

* From LeClerc *et al.* (1970)

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often used in conjunction with lime, tends to act against the high pH levels achieved by mixing sludge with lime and so inhibits lime's bactericidal properties (Table 84).

Conditioning of sludge with lime can significantly reduce densities of indicator and pathogenic bacteria in sludge. Farrell *et al*. (1974) have shown that conditioning of lime to pH 11.5 prior to dewaterng can reduce densities of fecal coliform bacteria, *Salmonella* spp. and *Pseudomonas aeruginosa* by two logs or more, with only limited regrowth of the fecal coliform bacteria and *Ps. aeruginosa* after 24 hr (Figure 32). Fecal streptococcus bacteria, however, were not seen to be removed significantly by this process. Doyle (1967) found no *Salmonella typhosa* in sludge samples limed so as to maintain a pH of 10.5 or greater after 24 hr, but he did find the pathogen in all samples the pH of which fell to pH 10.0 or less after 24 hr. Kampelmacher and van Noorle Jansen (1972) found lime conditioning to achieve reductions in densities of aerobic microorganisms, Enterobacteriaceae, and gram-positive bacilli and cocci on the order of two orders of magnitude and greater (Figure 33).

The bactericidal and virucidal properties of lime conditioning result from the high-pH environment that is created by mixing lime with sludge. The parameters of operation that affect the degree of microorganisms kill are pH and contact time at high pH. In current practice of lime conditioning at full-scale wastewater-treatment plants, neither parameter is monitored. Rather, lime is applied to sludge at pre-set doses that have been determined empirically to yield a sludge with good dewatering characteristics. Detention time prior to actual dewatering can vary greatly; it is not monitored, beyond assuring that some gentle mixing of sludge and lime is achieved before the sludge is fed to dewatering units. There is no assurance, therefore, that the bactericidal or virucidal effects of lime conditioning observed at laboratory and pilot scale have any parallel in full-scale operation.

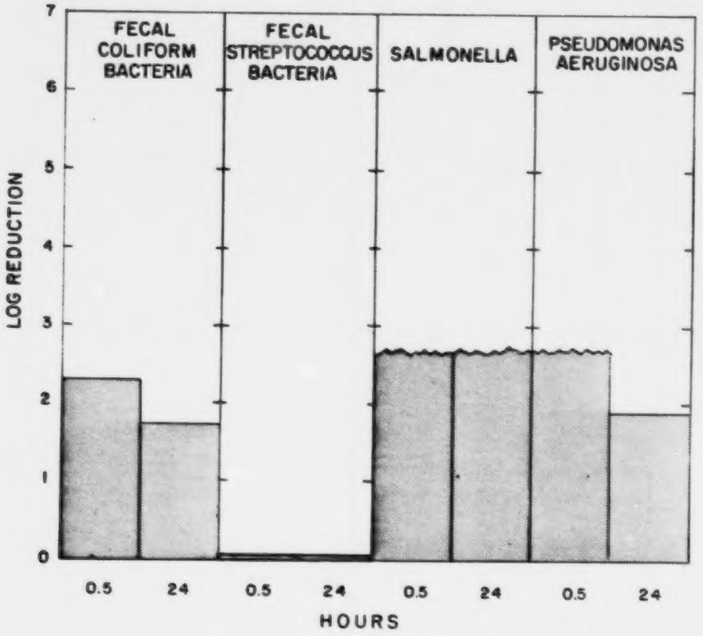

FIGURE 32. EFFECTS OF DETENTION TIME ON DENSITIES OF INDICATOR AND PATHOGENIC ORGANISMS IN SLUDGE TREATED BY LIME TO pH OF 11.5 AND DEWATERED BY VACUUM-FILTER LEAF.*

* FROM DATA OF FARRELL et al. (1974)

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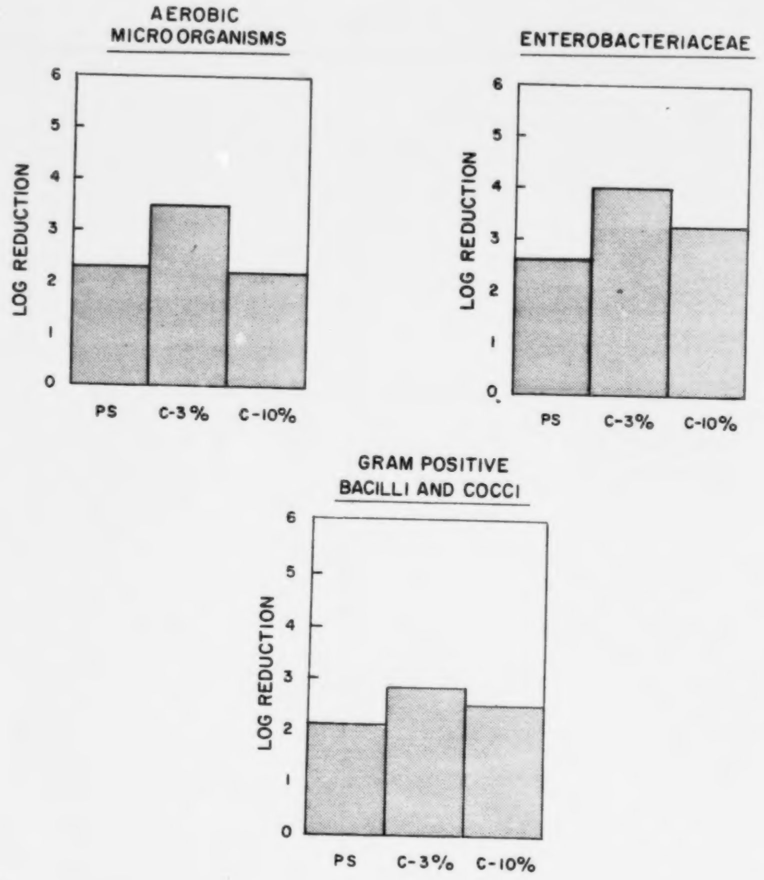

KEY:

PS - PRIMARY SLUDGE, CONDITIONED WITH 3-4 % LIME AND 0.5-2.5% FERRIC CHLORIDE, AND DEWATERED FROM 4 TO 25% SOLIDS.

C-3% COMBINED PRIMARY AND WASTE ACTIVATED SLUDGE, CONDITIONED WITH 3-4% LIME AND 0.5-2.5% FERRIC CHLORIDE, AND DEWATERED FROM 9 TO 26% SOLIDS.

C-10% COMBINED PRIMARY AND WASTE ACTIVATED SLUDGE, CONDITIONED WITH 10% LIME AND 2% FERRIC CHLORIDE AND DEWATERED FROM 8 TO 27-30% SOLIDS.

FIGURE 33. EFFECT OF SLUDGE TYPE AND LIME DOSE ON DENSITIES OF THREE TYPES OF MICROORGANISMS IN CONDITIONED/DEWATERED SLUDGE.*

* FROM DATA OF KAMPELMACHER AND Van NOORLE JANSEN (1972)

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SECTION 6

LITERATURE CITED

This section lists citations of all articles and reports that were used in this review. Abstracts of these articles and reports were included when available.

Following each citation, a Method Number refers the reader to Appendix B, where the methodology used by each author to enumerate bacteria, viruses and parasites is presented. Where the author did not document the analytical methodology used, "Not Specified" is listed.

1. Arther, R.G., and P.R. Fitzgerald. 1977. Viability of Nematode Ova Isolated from Anaerobically Digested Sludge. J. Parasit. 63 (suppl.): 69 (Abstr.).

   The Metropolitan Sanitary District of Greater Chicago is currently utilizing anaerobically digested sewage sludge as a soil conditioner. An assessment of the viability of nematode ova contained in this sludge was performed. Development of the ova to the larval stage occured in 58% of _A. lumbricoides_, 57% of _T. leonia_, 48% of _Toxocara_ sp. and 33% of _Trichuris_ sp. Nematode ova in sludge lagooned for 5 years was also capable of embryonation.

   Method No. 94.

2. Baxter, J.C., D. Johnson, W.D. Burge, and W.N. Cramer. 1980. Effects of Grazing Cattle on Forages Fertilized with Sewage Sludge. In preparation, EPA contract number 68-03-2210, U.S. Environmental Protection Agency, Cincinnati, Ohio. 190 pp.

   This study examined the effects of sewage disposal on agricultural land, and possible transmission of sludge contaminants into the food chain. Soils, forages, and cattle grazing a sewage sludge disposal site were examined for trace metals and refractory organics. To determine whether trace metal concentrations in tissues from the sludge exposed cattle were out of the ordinary, a group of cattle of known age and no exposure to sludge were examined. In addition, young feedlot cattle were fed a ration containing approximately 0, 4, or 12% sludge, which bracketed a range of sludge ingestion which might occur when cattle graze sludge amended pastures.

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Cattle grazing the sludge disposal site were healthy with no signs of pathology. The sludge exposed cattle showed higher concentrations of cadmium in kidney tissue when compared to one group of younger unexposed control animals, but no significant difference when compared to a larger group of unexposed control animals of known age.

Steers fed a feedlot ration amended with sewage sludge remained healthy. The sludge was shown to have no positive or negative effects on the general performance of the cattle other than to act as a diet diluent. Concentrations of cadmium, zinc, copper, lead, mercury, arsenic, and molybdenum were found to increase or decrease in one or more cattle tissues. These feeding studies demonstrated that there may have been interactions between dietary zinc, cadmium, and copper. Of 22 refractory organics examined, 4 were shown to increase in fat tissues due to sludge ingestion.

These studies showed that cattle would act as an effective screen against heavy metals entering the food chain from sewage sludges, and that incorporation of sewage sludges into the soil increased the effectiveness of this screen. If low metal sludges, such as the Metro Denver sludge, are incorporated into calcareous soils, there would appear to be little or no increase in risk of heavy metal movement into the human food chain.

Additional studies were conducted examining the die-off rate of pathogens in liquid sludge as the sludge dried in earthen drying basins. Analysis showed that as the sludge dried the numbers of fecal coliforms, total coliforms, salmonellae, f2 and T7 bacteriophage, and Ascaris ova decrease rapidly.

Method Nos. 1, 6, 20, 87

3. Berg, G., and D. Berman. 1980. Destruction by Anaerobic Mesophilic and Thermophilic Digestion of Viruses and Indicator Bacteria Indigenous to Domestic Sludges. Appl. and Environ, Micro., 39(2): 361-368.

In raw sludges and in mesophilically and thermophilically digested anaerobic sludges, large variations in numbers of viruses occurred over narrow ranges of numbers of fecal coliforms, total coliforms, and fecal streptococci, demonstrating that the bacteria were poor quantitative reflectors of the numbers of the viruses detected. Mesophilic and thermophilic digestion of anaerobic sludges destroyed all three indicator bacteria more rapidly than such digestion destroyed the viruses. The relative rates for the destruction of viruses, fecal coliforms, and fecal streptococci in the digested sludges were consistent over the 17-month study. Fecal coliforms were 7 to 8 times more sensitive than the viruses to mesophilic digestion and 9 to 10 times more sensitive to thermophilic digestion. Total coliforms were even more sensitive. The rates at which fecal streptococci were destroyed by mesophilic and thermophilic digestion of anaerobic sludges approaches those at which the

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viruses were destroyed by those processes; this suggested that the rates at which fecal streptococci in sludges are destroyed by mesophilic and thermophilic digestion may serve as useful indicators for the rates at which viruses in sludges are destroyed by these processes.

Method Nos. 4, 8, 11, and 64.

4. Bertucci, J.J., C. Lue-Hing, D. Zenz, and S.J. Sedita. 1977. Inactivation of Viruses During Anaerobic Sludge Digestion. J. Water Poll. Control. Fed., 49(7): 1642-1651.

   The possibility of increased virus burden to the environment caused by land application of digested municipal sludge was a major concern of the Metropolitan Sanitary District of Greater Chicago at its Fulton County Land Reclamation site in Central Illinois. Viruses in wastewater are adsorbed and concentrated by sludge floc during the activated sludge process, and are carried into the anaerobic digestion process. The virus inactivation potential of the digestion process was investigated by using five viruses and laboratory-scale digestion units. The viruses studied included coliphage MS-2, polio-virus-1, coxsackie viruses A-9 and B-4, and echovirus-11. Average individual virus inactivation rates were found to range from 74.9 to 97.1 percent after 24 hours to 93.7 to 99.9 percent after 48 hours.

   Method Nos. 19, 73

5. Bitton, G., S.R. Farrah, A.R. Overman, G.E. Gifford, O.C. Pancorbo, and J.M. Charles. 1980. Fates of Viruses Following Application of Municipal Sludge to Land. Unpublished manuscript.

   Method No. 55

6. Bond, J.O. 1958. The Risk of Ascaris Infestation from the Use of Human Sludge as Lawn Fertilizer. J. Fla. Med. Assn. 44(9): 964-967.

   Method No. 79

7. Bovay Engineers, Inc. 1975. Feasibility of Land Application for Spokane, Washington Wastewater Solids. Spokane, Washington.

   Method Nos. 4, 8 and 11

8. Brandon, J.R. 1978. Parasites in Soil/Sludge Systems. SAND77 - 1970, Sandia Laboratories, Albuquerque, N.M. 21 pp.

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Method No. 81

9. Brandon, J.R., W.D. Burge, and N.K. Enkiri. 1977. Inactivation by Ionizing Radiation of *Salmonella enteritidis* Serotype Montevideo Grown in Composed Sewage Sludge. Appl. and Environ. Microbiol., 33(4): 1011-1012.

S. enteritidis ser. montevideo were grown in composted sewage sludge to levels of approximately 10^9/g. These bacteria were found to be inactivated by ionizing radiation at approximately the same rate (30 krads/log) as *Salmonella* species in liquid digested sludge.

Method: Not specified

10. Brandon, J.R., and K.S. Neuhauser. 1978. Moisture Effects on Inactivation and Growth of Bacteria and Fungi in Sludges. In: Natl. Conf. on the Design of Municipal Sludge Compost Facilities, Chicago, Ill. pp. 48-53.

Method: Not specified

11. Brannen, J.P., D.M. Garst, and S. Langley. 1975. Inactivation of *Ascaris lumbricoides* Eggs by Heat, Radiation, and Thermoradiation. Sandia Laboratories, Albuquerque, N.M., SAND75-0163. 26 pp.

This report describes experimentation to determine the effects of heat, radiation, and thermoradiation on the suppression of embryonation of *Ascaris lumbricoides* ova. Heat effects were observed at a minimum temperature of 51°C and radiation effects at doses in excess of 15 krads of ionizing gamma radiation. Thermoradiation at 47°C suppressed embryonation at less than half the total dose required by radiation.

Method No. 81

12. Burge, W., D. Colacicco, W. Cramer, and E. Epstein. 1978. Criteria for Control of Pathogens During Sewage Sludge Composting. In: Nat. Conf. on the Design of Municipal Sludge Compost Facilities, Chicago, Ill. pp. 124-129.

Method: Not specified

13. Burge, W.D., and W.N. Cramer. 1974. Destruction of Pathogens by Composting Sewage Sludge. Maryland Environmental Service, Annapolis, Md., and Water Resources Management Administration, Washington, D.C. 46 pp.

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Method Nos. 1, 6, 20, and 26

14. Burge, W., P. Marsh, and P. Millner. 1977. Occurrence of Pathogens and Microbial Allergens in the Sewage Sludge Composting Environment. In: Proc. of Natl. Conf. on Composting of Municipal Residues and Sludges, Silver Springs, Md. pp. 128-134.

Method: Not specified

15. Cliver, D.O. 1975. Virus Association with Waste Water Solids. Environ. Lett., 10(3): 215-223.

The solids produced at an urban wastewater treatment plant, tested semi-quantitatively, almost all contained human intestinal viruses. Reoviruses and five or more types of enteroviruses were present. Sludge, digested anaerobically at 30° - 32°C, and grit contained measurable levels of viruses. Until reliable means of inactivating the viruses have been developed and implemented, great care should be taken in disposing of these solids.

Method No. 61

16. Connery, J. 1978. Health Implications of Alternatives: Ocean Disposal, Incineration, Land Application, and Land Filling. Draft Position Papers for the Workshop on the Health and Legal Implications of Sewage Sludge Composting. Energy Resources, Inc., Cambridge, Mass. Chpt. 9.

Method: Not specified

17. Cooke, W., and P. Kabler. 1955. Isolation of Potentially Pathogenic Fungi from Polluted Water and Sewage. Public Health Report, 70(7): 689-694.

Method No. 48

18. Cooke, M.B., E.L. Thackston, and G.W. Malaney. 1978. Reducing Coliform and *Salmonella* Bacteria During Anaerobic Digestion. Water & Sewage Works, 125(1): 50-53.

Raw sludge from the primary settling tanks and digested sludge were sampled twice in Nov. and once in Dec. 1975 at 3 sewage treatment plants near Nashville, Tennessee. Coliform densities were determined by the multiple-tube fermentation technique, Salmonellae concentrations by the Alley-Malaney membrane filter technique. With 1 unexplained exception, there was a statistically significant reduction in both microorganism

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concentrations at each site on each sampling date. Statistical tests proved the reduction to be due to the anaerobic digestion process. The coliform concentration was reduced by a factor of 10^4 at the 2 Murfsboro sites, but only by 10^2-10^3 at Dry Creek. Salmonellae were reduced by a factor of 10^2 at Dry Creek, but by 10^3-10^4 at the other sites. These differences may be due to the average detention time, which was only 9 days at Dry Creek, compared to 38 and 50 days in the 2 Murfsboro digesters. Coliform densities in the digested sludge were 10^4-10^6 organisms/100 ml of sludge, while Salmonellae concentrations were 10^4-10^5/100 ml. Whether or not these concentrations of Salmonellae pose health hazards must be determined by research into their longevity in the soil and possible modes of transmission to man in numbers high enough to cause disease. It is important to realize that even the 99.9% reduction in Salmonellae reported in this study is meaningless if the actual final concentration is 10^4/100 ml while a dose of 10^3/100 ml is enough to produce disease.

Method Nos. 1, 32

6-1/2"

19. Cooper, R.C., and C.G. Golueke. 1979. Survival of Enteric Bacteria and Viruses in Compost and its Leachate. Compost Sci./Land Util., 20(2): 29-35.

A study conducted at the University of California indicates that composting may not be an effective means of disinfecting sewage. The chemical and biological properties of the leachate were determined in large and small scale studies. Virus levels were too difficult to measure for conclusive results, but bacterial studies showed that despite a sharp decline in populations, at the end of 46 d the actual numbers of bacteria ranged from 1.31×10^5 to 1.31×10^7/GDW of compost. Frequency of turning did not affect the die-off rate of test organisms.

Method Nos. 1, 6, 10, and 63

20. Counts, C.A., and A.J. Shuckrow. 1974. Lime Stabilized Sludge: Its Stability and Effect on Agricultural Land. Nat. Environ. Res. Center, U.S. Environ. Protection Agency, Cincinnati, Oh. 87 pp.

Method Nos. 8, 12, and 30

21. Cram, E.B. 1943. The Effect of Various Treatment Processes on the Survival of Helmith Ova and Protozoan Cysts in Sewage. Sewage Works, 15(11): 1119-1138.

Method: Not specified

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22. Cramer, W.N. 1975. Destruction of an Indicator Virus During Composting of Sewage Sludge. In: Meeting on Agronomic Research For Food, Knoxville, Tenn. 15 pp.

Method No. 20

23. Cramer, W.N., and W.D. Burge. 1977. Inactivation of F-2 Bacterial Virus by Ammonia. Abstracts of the Annual Meeting American Society Microbiology, 77, 255 pp.

Method: Not specified

24. Cramer, W.N., and W.D. Burge. Comparative Virucidal Capabilities of Ammonia and Halogens. Abstract, Maryland Environmental Service, Annapolis, Md., and USDA, Beltsville, Md.

Method: Not specified

25. Danielsson, M.L. 1977. *Salmonella* in Sewage and Sludge: Serological Profiles of Isolates, Their Removal and/or Survival in Relation to Potential Health Hazards to Man and Animals. Acto. Vet. Scand. Suppl., 65: 1-126.

The effectiveness of different methods for sewage and sludge treatment plants in Sweden were studied. The frequencies of *Salmonella* in sewage treatment plants were higher with pre-precipitation than with post-precipitation. Chlorination reduced viable counts of indicator bacteria but did not guarantee that the sewage was free from *Salmonella*. The survival of *S. enteritidis* was longer in sludge incubated anaerobically than aerobically. *S. enteritidis* appeared to survive for a long time in recipient watercourses. There are several possible health hazards produced by presence of *Salmonella* in sewage and sludge. Drinking water and pastures contaminated by flooding of recipient watercourses are the most common source for infected animals in Sweden. Aquatic fauna may also be infected by *Salmonella*-contaminated recipient watercourses. The risk of *Salmonella* infection for bathers seems to be minimal, unless water is contaminated with *S. typhi* or *S. paratyphi*. Contaminated irrigation water or water used in food preparation may also be a source of *Salmonella* infection. A study of the risk of infection to sewage treatment plant workers should be considered. The use of contaminated sludge as fertilizer presents the same risk as the use of contaminated water for irrigation.

Method No. 31

PAGE NUMBER
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26. Dean, R. B., and J.E. Smith. 1973. The Properties of Sludges. In: Proceedings of Joint Conference on Recycling Municipal Sludges and Effluents on Land, U.S. Environmental Prot. Agency, U.S.D.A., and National Assoc. State Univ. and Land Grant Colleges, Champaign, Ill. pp. 39-47.

Method: Not specified

27. Doyle, C. 1967. Effectiveness of High pH for Destruction of Pathogens in Raw Sludge Filter Cake. J. Water Poll. Control Fed., 39(8): 1403-1409.

At the sewage works of Greenwood, S.C., which treats both domestic and industrial sewage, crude sludge is conditioned with lime and ferric sulphate and dewatered by vacuum filtration before disposal on land as soil conditioner. Occasional complaints about odours from the disposal area suggested that conditions might be favourable for survival of pathogenic bacteria; such odours were eliminated when the dose of lime was increased, and experiments were, therefore, carried out on the effect of high pH values on the survival of *Salmonella typhosa* and coliform bacteria in the filter cake. The results, summarized in a table, showed that survival of *Salmonella typhosa* was inversely related to the pH value; complete destruction of the pathogen was obtained by treatment with excess lime to give a pH value of at least 12. The coliform group appeared to be more resistant to pH value than *Salm. typhosa* and is not considered to be a satisfactory indicator of the safety of the sludge.

Method Nos. 1, and 21

28. Dudley, J.D., M.N. Guentzel, M.J. Ibarra, B.E. Moore, and B.D. Sagik. 1980. Enumeration of Potentially Pathogenic Bacteria From Sewage Sludges. Appl. and Environ. Microbiol., 38(1): 118-126.

To ascertain the health risks that may be posed by the land application of sewage sludges, a scheme was devised to determine the types and numbers of pathogenic and potentially pathogenic bacteria present in sludges. A processing treatment was adapted to sludge to give a homogenate which yielded the greatest numbers of viable bacteria. Conventional methods were successful in enumerating *Klebsiella*, *Staphylococcus*, gram-negative enteric bacteria, and commonly used indicator organisms. Modifications of conventional methods improved the enumeration of *Salmonella*, *Mycobacterium* sp., fluorescent *Pseudomonas* sp., and *Clostridium perfringens*. However, *Shigella* methodology yielded only one isolate. Utilizing the proposed scheme, the population densities of these organisms were estimated in three domestic wastewater sludges. In light of these results, the potential impact of land application of sewage sludges is discussed.

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Method Nos. 5, 9, 13, 16, 24, 35, 36, 41, 42, 43, 44, 45, 46, and 47

29. Eisenhardt, A., E. Lund, and B. Nissen. 1977. The Effect of Sludge Digestion on Virus Infectivity. Water Res., 11(7): 579-582.

The rate of inactivation of coxsackievirus B3 during anaerobic sludge digestion was found to be 2 log units in 24 h at pH 7.0 and 35°C. The rate indicates that virus inactivating factor(s) were produced in the digestion. The virus inactivation was enhanced if the digester sludge was pasteurized at 60°C for 1 h. The reason why digesters at sewage treatment plants do not completely inactivate virus is discussed in terms of the continuous nature of digester operation.

Method No. 77

30. Epstein, E. 1978. Technical Aspects of Composting as a Disinfection Process: An Overview. In: Draft Position Papers for the Workshop on the Health and Legal Implications of Sewage Sludge Composting. Energy Resources Co., Inc., Cambridge, MA. Chpt 1.

Method: Not specified

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31. Epstein, E., G.B. Wilson, W.D. Burge, D.C. Mullen, and N.K. Enkiri. 1976. A Forced Aeration System for Composting Wastewater Sludge. J. Water Poll. Control Fed., 48(4): 688-694.

Studies were conducted on composting wastewater sludge to prevent offensive odors. A forced aeration system was developed for composting anaerobically digested, mixed raw (primary and activated secondary undigested), and digested sludge. Because temperatures in the aerated pile were higher than those in a windrow composting system, there was more extensive elimination of total coliforms, fecal coliforms, and salmonellae. Thermophilic temperatures above 40°C and often exceeding 70°C were maintained for 14 to 26 days. Oxygen levels for composting in the pile were in the range of 10 to 15 percent. The odoriferous gases were drawn from the pile by suction and deodorized by passing through a pile of screened compost.

Method Nos. 3, 7, and 28

32. Farrell, J.B. 1979. Processes to Significantly Reduce Pathogens: Aerobic Digestion. Inhouse Report submitted to E. Lazar, U.S.E.P.A., Cincinnati, Ohio. 30 July 1979.

Method: Not specified

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33. Farrell, J., J. Smith, S. Hathaway, and R. Dean. 1974. Lime Stabilization of Primary Sludges. J. Water Poll. Control Fed., 46(1): 113-122.

An investigation verified the fact that lower sludge solids may be obtained when alum and iron are added to the primary clarifier. The addition of lime to alum and iron chemical/primary sludges increased the vacuum filter yield to reasonable rates. Sand bed dewatering was found not to be adversely affected by the lime addition. The addition of lime was found not to make the sludges chemically stable. The pH was found to fall eventually, and the surviving bacteria could return when conditions were favorable to their growth. When the pH of the the sludge was raised to 11.5 by the application of lime, the bacterial hazard of the material was reduced to near zero. Long-term effects of lime addition were not determined.

Method Nos. 8, 11, and 30

34. Feige, W.A., E.T. Oppelt, and J.F. Kreissl. 1975. An Alternative Septage Treatment Method: Lime Stabilization/Sand-Bed Dewatering. EPA 600/2-75-036, U.S. Environ. Protection Agency, Cincinnati, Oh. 52 pp.

Method Nos. 6, 10, and 30

35. Fenters, J., J. Reed, C. Lue-Hing, and J. Bertucci. 1979. Inactivation of Viruses by Digested Sludge Components. J. Water Poll. Control Fed., 51(4): 689-694.

A study was conducted to determine rates of inactivation of three enteroviruses seeded into anaerobically digested sludge supernatant. Ammonia, a major chemical component of sludge, also was evaluated at various concentrations for its effect on virus infectivity. Data indicated that virus inactivation rates varied in proportion to temperature over a range of 20° to 35°C and that sludge supernatant had a greater impact on virus reduction than control Medium 199. In addition, the rate of Poliovirus 1 inactivation was increased in proportion to the concentration of ammonia over the range of 50 to 1500 mg/l. Antibiotics added to sludge supernatants did not affect virus inactivation rates.

Method No. 75

36. Fitzgerald, P., and R. Ashley. 1977. Differential Survival of *Ascaris* Ova in Wastewater Sludge. J. Water Poll. Control Fed., 49(7): 1722-1724.

Ova of *Ascaris lumbricoides suum* held at 38°C in liquid sludge, in a simulated anaerobic digester for 25 days, were less susceptible to the effects of the environment than ova held at 38°C in physiological

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saline. Ova failed to embryonate when held at 38°C for 21 or 25 days in sludge or physiological saline. However, after removal from contact with sludge and subsequent exposure to air and 1 percent formalin for 58 days at 22°C, up to 90 percent of the ova embryonated. Oocysts of *Eimeria stiedai* exposed to the same conditions as the *Ascaris* ova were all killed within 5 days. Sludge from four different systems were tested. One of the four was lethal to the ova. The lethal factor was not determined but the results reemphasize the highly variable nature of sludge. This study shows that *Ascaris* ova recovered from sludge, that appear dead, may be healthy and viable when given time and the proper environment in which to embryonate.

Method No. 85

37. Fitzgerald, P.R., and T.B.S. Prakasam. 1978. Survival of *Trichinella spiralis* Larvae in Sewage Sludge Anaerobic Digesters. Parasitol. J., 64(3): 445-447.

The survival of some bacteria, viruses, protozoans, and helminths through the sewage digestion process has been a question of considerable concern among researchers throughout the world. Among the most resistant organisms are some of the pathogenic roundworms and tapeworms. Encysted larvae of *Trichinella spiralis* are sometimes present in animal tissues discarded as waste from slaughterhouses, restaurants or other sources. In experimental anaerobic sewage digesters, encysted larvae of *T. spiralis*, in rat muscle, were able to survive a maximum of 96 hr in a "batch" digester. In a digester "fed" daily with small numbers of encysted larvae, "draw-off" remained infective for white rats throughout a 16-day experimental period. Potentially infective material could be present when there is continuous "draw-off" from the anaerobic digesters.

Method No. 90

38. Fox, J.C., and P.R. Fitzgerald. 1977. Parasite Content of Municipal Wastes From the Chicago Area. J. Parasitology. 63 (suppl.): 68-69.

Method: Not specified

39. Fuller, J.E., and W. Litsky. 1950. *Escherichia coli* in Digested Sludge. Sewage and Indust. Wastes, 22(7): 853-859.

A study was made on the viability of *Escherichia coli* in drying digested sludge to determine the suitability of sludge for use as fertilizer. For different samples of sludge, the ratio between *Esch. coli*, *Aerobacter*, and Intermediate types was determined. Wet sludge from different levels in the digestion tank contained considerable numbers of *Esch. coli*; the ratio of this organism to *Aerobacter* decreased and the

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ratio of intermediate organisms to Aerobacter increased during digestion. Digested sludge was filtered and dried under controlled conditions at 22°C and at 37°C. At both temperatures the Most Probable Number of coliform organisms decreased but the decrease was more rapid at the higher temperature. The numbers of Esch. coli decreased slowly at 37°C, and the organism disappeared after 56 days; at 22°C the numbers decreased rapidly and the organism disappeared after 14 days. After storage for 80 days the total number of bacteria was still high but the M.P.N. of coliform bacteria was almost negligible. It appeared that temperature, content of moisture, pH value, bacteriophage, and antibiotic agents were not determining factors in the disappearance of Esch. coli. Experimental evidence indicated that the organism disappeared because of competition from other micro-organisms better suited to survival under the existing conditions. It is considered that the absence of Esch. coli from dried sludge would indicate that pathogenic intestinal organisms such as typhoid and dysentery bacilli would also be absent and that sludge would be safe for use as fertilizer. It cannot however be assumed that the absence of Esch. coli would indicate that safety from other diseases such as poliomyelitis and tuberculosis can be assured.

Method No. 2

40. Gaby, W.L. 1975. Evaluation of Health Hazards Associated with Solid Waste/Sewage Sludge Mixtures. EPA/670/2-75-023, U.S. Environ. Protection Agency. 56 pp.

The report summarizes and evaluates the health hazards associated with municipal solid waste/sewage sludge composting by the windrow composting process. The occurrence and survival of pathogens, parasites, and indicator bacteria at various stages during the composting process are described. The study shows that windrow temperatures of 120°F to 167°F (49°C-74°C) maintained for at least seven days destroy pathogens and human parasites. Dog parasitic ova, however, remain intact 35 days after exposure. Considerable variation in the temperature is found at the top and bottom two inches of the windrow indicating the proper turning of the compost is essential to ensure destruction of pathogens and parasites. It is concluded that a properly composted solid waste or solid waste/sewage sludge mixture is microbiologically acceptable as a soil conditioner for gardens, farms, and lawns, or for filling areas of erosion without creating health hazards.

Method Nos. 1, 6, 22, 49, and 91

41. Glass, J.S., R.J. Van Sluis, and W.A. Yanko. 1978. Practical Method for Detecting Poliovirus in Anaerobic Digester Sludge. Appl. and Environ. Microbiol., 35(5): 983-985.

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Method No. 69

42. Glathe, I., W.F. Hirte, K. Krannich, and F. Moeller. 1972. Model Investigations on the Question of Disinfection of Waste from Settlements by Composting. Z. Gesamte Hyg. Grenzgeb., 18(1): 1-6.

Method No. 21

43. Gray, K.R., K. Sherman, and R.J. Biddlestone. A Review of Composting, Part 1: Process Biochemistry. June: 32-36 via Epstein, E. 1978. Technical Aspects of Composting as a Disinfection Process: An Overview. In: Draft Position Papers for the Workshop on the Health and Legal Implications of Sewage Sludge Composting. Energy Resources Co., Inc., Cambridge, MA. Chpt. 1.

Method: Not specified

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44. Hays, B. 1977. Potential for Parasitic Disease Transmission with Land Application of Sewage Plant Effluents and Sludges. Water Res., 11(11): 583-595.

Land application of sewage plant effluents and sludges is being considered by planners because of environmental problems arising from alternative approaches and as a means of recovering the soil enrichment qualities of sewage effluents and sludges. A review of the literature shows that primary and secondary treatment, anaerobic sludge digestion, and routine disinfection (chlorine or ozone) do not destroy completely parasitic forms. Because of their weight most cysts and eggs are found in the sludge. Sludge digestion does destroy protozoan cysts but not all metazoan eggs. These cysts and eggs are extremely resistant in field conditions and some can persist infective for long periods. Documented disease transmission to humans from land application programs is related only to use of raw, not treated, sewage on land. Some animal disease transmission has been found related to untreated and partially treated sewage. Those who have examined sewage plant effluents and sludges for parasitic cysts and eggs have found them present. The most commonly reported species are: *E. histolytica*, *Ascaris lumbricoides*, *Trichuris trichiura*, *Hymenolepis* spp, *Taenia* sp, *Enterobius vermicularis*, *Ancylostoma* and *Necator*. Destruction of all pathogenic forms can be assured by adequate heating of material to pasteurization temperatures of 60°C or more for 30+ min. Direct heating or composting can accomplish this. Parasitic disease transmission can be reduced by controlling the types of land to which these sewage plant products are applied. Parasitic disease transmission should be considered in developing the technology and/or management approaches to land application of sewage plant products for soil enrichment purposes.

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Method: Not specified

45. Hess, E., and C. Breer. 1975. The Sanitary Effect of Gamma Irradiation on Sewage Sludge. In: Radiation for a Clean Environment, Intern. Atomic Energy Agency, Vienna. pp. 203-208.

Method: Not specified

46. Heukelekian, H. and M. Albanese. 1956. Enumeration and Survival of Human Tubercle Bacilli in Polluted Waters. II. Effect of Sewage Treatment and Natural Purification. Sewage and Indust. Wastes 28(9): 1094-1102.

Experiments were made to determine the effect of sewage treatment and self-purification of polluted waters on the numbers of tubercle bacilli. The bacilli were determined after preliminary treatment of the sample with either chlorine or Bradosal (see preceding abstract). It was found that there were significant numbers of tubercle bacilli in the sewage from the 3 sanatoria studied. Sedimentation, treatment in septic tanks, biological filtration, and the activated-sludge process were all ineffective in removing tubercle bacilli from sewage. The pathogen was removed, however, by chemical coagulation with ferric chloride and by intermittent slow sand filtration. The organism was destroyed by chlorination to give a concentration of residual chlorine of 2 ppm after a period of contact of 30 min, or of 1 ppm after a period of contact of 1 hour. Tubercle bacilli in primary and secondary sewage sludges were not destroyed by anaerobic digestion or by air drying of sludge in open sand beds. The survival of tubercle bacilli in surface waters was also investigated under laboratory conditions. Tubercle bacilli did not disappear completely from fresh water stored for 6 days nor from saline water stored for 24 hours. The rate of decrease in numbers of organisms was greater in saline water than in fresh water.

Method No. 39

47. Hinsley, T.D. 1974. Agricultural Benefits and Environmental Changes Resulting from the use of Digested Sludge on Field Crops. EPA/530/SW-30d.1, U.S. Environ. Protection Agency. 404 pp.

The effects of digested sludge application on the chemical composition of soil, plant, and water samples from a large field lysimeter facility are discussed. Specific hygienic aspects of digested sludge were also investigated and, it was found that viruses are not likely to survive the heated anaerobic digester environment and, although digested sludge contains large populations of fecal coliform bacteria, these organisms die away rather rapidly during storage and after spreading on the soil. Results from green house and field studies indicate that several crop plants show favorable growth responses when fertilized with digested

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sludge, however, concentration levels of several chemical elements in soils are increased above native amounts and are also increased in plant tissues. As long as digested sludge application rates do not exceed those which will result in unacceptable concentration levels of NO_3-N in drainage or groundwaters, sludge of the quality employed in the study can be safely used to increase the production of good quality crops.

Method No. 8

48. Horvath, R.W. 1978. Operating and Design Criteria for Windrow Composting of Sludge. In: Proc. of the Nat. Conf. on Design of Municipal Sludge Compost Facilities, Chicago, Ill. pp. 88-95.

Method: Not specified

49. Hurst, C., S. Farrah, C. Gerba, and J. Melnick. 1978. Development of Quantitative Methods for the Detection of Enteroviruses in Sewage Sludges During Activation and Following Land Disposal. Appl. and Environ. Microbiol., 36(1): 81-89.

Activated sewage sludge solids were collected by centrifugation, and elution of the solid-associated virus was accomplished by mechanical agitation in glycine buffer at pH 11.0. Eluted viruses were concentrated either onto an aluminum hydroxide floc or by association with a floc which formed de novo upon adjustment of the glycine eluate to pH 3.5. Viruses which remained in the liquid phase after lowering the pH of the glycine eluate were concentrated by adsorption to and elution from membrane filters. The method included high pH glycine elution and subsequent low pH concentration. The recovery from activated sludge was 80 for poliovirus type 1, 68% for echovirus type 7, and 75% for coxsackievirus B3. Reductions of enterovirus titers per gram (dry weight) of solids were modest during sludge activation but increased to a rate of 2 $\log_{10}$/wk after land disposal.

Method No. 55

50. Iacoboni, M. 1977. Pathogen Inactivation During Sludge Composting. Internal Reports of County Sanitation Districts of Los Angeles County, November and December, 1977.

Method: Not specified

51. Iacoboni, M., and T. LeBrun. 1977. Pathogen Inactivation During Windrow Composting. Internal Reports of the County Sanitation Districts of Los Angeles County, May and June, 1978.

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Method: Not specified

52. Iacoboni, M., and R. Livingston. 1977. Pathogen Inactivation During Sludge Composting. Internal Reports of the County Sanitation Districts of Los Angeles County. July, October, December 1977.

Method: Not specified

53. Jewell, W.J., R.M. Kabrick, and J.A. Spada. 1980. Autoheated Aerobic Thermophilic Digestion with Air Aeration. In Press: Municipal Environmental Research Lab, U.S. Environ. Protec. Agency, Cincinnati, Ohio. R804636.

A full scale study (28.4 m^3 reactor) demonstrated that simple self-aspirating aerators (ambient air) could achieve high oxygen transfer efficiencies and thereby allow conservation of the heat of oxidation to achieve autoheating to high temperatures. Continuous feed operation of a one stage digestion system utilizing a feed of primary and waste activated sludge (3% - 6% total solids) resulted in autoheating of reactor temperatures ranging from 45°C to 65°C.

The relationship between process variables and the autoheating phenomena were examined at full-scale and bench scale levels. The process variables included organic loading rate and dissolved oxygen concentration. It was observed that intermediate loading rates (12-15 kg TS/m^3 reactor-day) and low dissolved oxygen residuals ($\leq$ 1 ppm) allowed maximum temperature development.

Two different aerators were tested and were found to achieve oxygen transfer efficiencies exceeding 20% at reactor temperatures that often exceeded 60°C. Operational problems associated with these kinds of aerators as well as with the other equipment on the thermophilic digestion facility were identified and examined.

The potential of the autoheated thermophilic digester to inactivate pathogens was investigated. Virus inactivation was 100% in most cases, with bacterial and parasite indicator counts less than those found in the full scale mesophilic anaerobic digester effluent.

Dewaterability of the autoheated thermophilic digester effluent deteriorated at all loading conditions studied. The aerobic thermophilic digestion process appears to increase the solubility of various heavy metals such as cadmium.

A computer model was developed from the full scale data for predicting the reactor temperature under given loading conditions.

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Method Nos. 4, 8, 11, and 21

54. Jones, M.F., W.L. Newton, S.R. Weibel, H.B. Warren, M.L. Steinle, and W.B. Figgat. 1947. The Effects of Sewage Treatment Processes on the Ova and Miracidia of *Schistosoma japonicum*. Nat. Inst. of Health Bull., 189: 137-172.

Method: Not specified

55. Kampelmacher, E.H., and L.M. van Noorle Jansen. 1972. Reduction of Bacteria in Sludge Treatment. J. Water Poll. Control Fed., 44(2): 309-313.

The filtration of sludge, carried out with the aid of ferric chloride or ferrous sulfate and lime and with a vacuum filter, yields an end product in which the number of bacteria is reduced considerably. In sludge treatment plants at Apeldoorn and Mierlo, the Netherlands, a reduction of the order of two decimals was found for the aerobic bacteria and of the order of two to three decimals for the enterobacteriaceae. In the plant in Maastricht, the reduction in the number of aerobic bacteria was of the order of three decimals and that of enterobacteriaceae, of four decimals. In all three plants, *Salmonella* contamination of the dry sludge was significantly less than that of the wet sludge.

Method Nos. 17, and 23

56. Kawata, K., W.N. Cramer, and W.D. Burge. 1977. Composting Destroys Pathogens in Sewage Solids. Water and Sewage Works, 124(4): 76-79.

Method: Not specified

57. Keller, P. 1951. The Influence of Heat Treatment on the Ova of *Ascaris lumbricoides* in Sewage. J. and Proc. Inst. Sewage Purif., pt 1: 100-109.

Thermophilic digestion of sewage sludge on a laboratory scale has been tested experimentally as a means of destroying or inactivating the ova of parasitic forms and the cysts of amoebae parasitic to man, which are usually found in sewage sludge during practically all stages of treatment for disposal. It has been found that thermophilic digestion for a minimum period of twenty-four hours destroys all parasitic ova and cysts. Since thermophilic digestion as such, however, entails certain disadvantages, the effect of heat treatment on sewage sludge after digestion at mesophilic temperatures has been investigated. It has been found that prolonged exposure of sewage sludges to a temperature of 37°C completely inactivates the ova of *Ascaris lumbricoides* which served as the index organism. The process as such, however, is impracticable,

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since the period of time required for complete destruction would be too long. It has been found, however, that if normally digested sewage sludge or raw sewage sludge is heated for a period of two hours at 55°C, complete destruction of all parasitic ova and cysts usually met with in sewage occurs. Heating sewage sludges to higher temperatures decreases the period of exposure necessary, but decreases at the same time the ease of filtration of the sludge and imparts an objectional odour to it which makes the use of drying beds, or open storage of the liquid sludge impossible. The effect of the Porteous process on the ova of *A. lumbricoides* has also been investigated, but incorporation of the process into existing conventional sewage treatment does not appear to be warranted for various reasons. The influence of heat treatment on pathogenic bacteria and viruses has not been investigated.

Method No. 93

58. Keller, P., and C. G. Hide. 1951. Sterilization of Sewage Sludges; Incidence and Relative Viability of *Ascaris* Ova at Sewage Disposal Works in Johannesburg Area. S. African Med. J. 25: 338-342.

Method No. 93

59. Kelly, S., and W.W. Sanderson. 1959. The Effect of Sewage Treatment on Viruses. Sewage and Indust. Wastes, 31(b): 683-689.

Studies were conducted to determine the types of sewage treatment that destroy viruses under operating conditions. Types of treatment included primary sedimentation in both Imhoff tanks and mechanically cleaned sedimentation tanks, secondary treatment by trickling filters and by activated sludge, clarification with chlorination, and sludge digestion. The origin of the various materials sampled is described. Of 140 samples collected in 1957, over 166 strains of viruses were isolated. The indications were that existing sewage treatment methods may not destroy viruses. Some of the treatment reduced the concentration of infectious particles only slightly. Effluent from an activated-sludge treatment did not contain viruses, and they were not found in the Imhoff tank or digested sludge. The results are critically discussed in the light of the existing literature on the subject.

Method No. 65

60. Kenner, B.A., and H.P. Clark. 1974. Detection and Enumeration of *Salmonella* and *Pseudomonas aeruginosa*. J. Water. Poll. Control Fed., 49(9): 2163-2171.

Method No. 30

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61. Kenner, B.A., G.K. Dotson, and J.E. Smith. 1971. Simultaneous Quantitation of *Salmonella* Species and *Pseudomonas aeruginosa*. I. Polluted Water. II. Persistence of Pathogens in Sludge Treated Soils. III. Analysis of Waste Treatment Sludges for Salmonellae Species as a Surveillance Tool. U.S. Environ. Protection Agency, Cincinnati, Oh. 43 pp.

The report is divided into three sections. In the first a practical sensitive quantitative method for the isolation and enumeration of *Salmonellae* and pyocyanogenic *Ps. aeruginosa* from wastewaters, treatment effluents, streams, stormwater runoff, and sludges, in all seasons of the year, is described. Next the persistence of *Salmonella* species, *Pseudomonas aeruginosa*, and fecal coliforms in soils treated with various types of municipal sludges is reported. In the last report it is suggested that a more complete and useful surveillance of salmonellosis for large urban areas can be attained by analysis monthly of municipal primary and secondary waste treatment plant sludges.

Method Nos. 8, and 30

62. Krige, P. R. 1964. A Survey of the Pathogenic Organisms and Helminthic Ova in Composts and Sewage Sludge. J. and Proc. Inst. Sewage Purif., Part 3, p. 215-220.

Tabulated results are given summarizing research carried out by the South African Council for Scientific and Industrial Research on the survival of pathogenic organisms and helminth ova in sewage sludge and composts; the methods of examination used are indicated. Analysis of matured compost from various municipalities in South Africa showed that there was no direct relation between the survival of *Esch. coli* and the survival of *Ascaris* ova. In aerobic systems, such as the mechanical composting plant operated experimentally by C.S.I.R., the numbers of Esch. coli were higher than in anaerobic systems. With proper operation and the correct period of maturation, the C.S.I.R. mechanical plant produced a compost consistently free from *Ascaris* ova, and poliomyelitis virus was eliminated in the stabilization plant while the materials were passing through the drum. From the results, it is concluded that compost produced from sewage sludge under controlled conditions does not constitute a danger to health.

Method Nos. 83, and 84

63. Krogstad, O., and R. Gudding. 1975. The Survival of Some Pathogenic Microorganisms During Reactor Composting. Acta Agri. Scand., 25: 281-284.

Method Nos. 14, and 33

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64. Kruse, C. W. 1977. Sludge Disinfection. In: Evaluation of Current Developments in Municipal Waste Treatment, W. C. Remini, ed., Baltimore, Md. pp. 7-14.

Method: Not specified

65. Laconde, K., R. Lofy, and R. Stearns. 1978a. Municipal Sludge Agricultural Utilization Practices; An Environmental Assessment, Vol. I. EPA 530-SW-709, U.S. Environ. Protection Agency. 150 pp.

An environmental assessment was performed at nine study sites across the United States to investigate the effects of utilizing municipal wastewater treatment plant sludge for agricultural purposes. The sites represented a wide range of sludge applications rates, sludge characteristics, cropping practices, soils, population densities, and climatological and geographical conditions. The assessment included evaluative criteria such as chemical, physical, and microbiolgical constituents of sludges; of heavy metals, pesticides, nutrients, bacteria and parasites; plant characteristics including heavy metal and pesticide uptake, and potential contamination by bacteria and parasites; public attitudes; landspreading costs (capital and operational); current and past operating procedures; and management practices at each of the nine sites.

Method Nos. 6, 10, 21, and 93

66. Laconde, K., R. Lofy, and R. Stearns. 1978b. Municipal Sludge Agricultural Utilization Practices; An Environmental Assessment, Vol. II. EPA/530/SW-156C, U.S. Environ. Protection Agency.

Method Nos. 6, 10, 21, and 93

67. LeClerc, H., A. Perchet, C. Savage, S. Andrieu, and R. Nguematcha. 1970. Microbiological Aspects of Sewage Treatment. In: Advances in Water Pollution Research, Proc. of the 5th Intl. Conf., San Francisco and Hawaii, S. H. Jenkins, ed. pp. I23/1-9.

Method Nos. 18, 27, 38, and 57

68. Lund, E. 1970. Observations on the Virus Binding Capacity of Sludge. In: Advances in Water Pollution Research, Proc. of the 5th Intl. Conf., San Francisco and Hawaii, S.H. Jenkins, ed. pp. I-24/1-5.

Observations accumulated over a number of years on the recovery of viruses from sewage and treated effluents by the phase separation method, and by other methods, are described. Relative recoveries of viruses from digester sludge, sewage influents, and treated effluents are com-

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pared. Data suggesting that viruses in sewage and sludge are bound to large particles are discussed.

Method No. 60

69. Lund, E. 1973. The Effect of Pre-treatments on the Virus Contents of Sewage Samples. Water Res., 7(6): 873-879.

By isolations of virus from sewage and sludges samples, which had been fractionated by centrifugation at 9000 rev min^{-1}, it was found that virus may be particle bound and recoverable in the precipitates. An exception was the secondary sludge of the activated sludge treatment. It was thus indicated that removal of a part of a sewage or sludge sample before examination may make true quantitative estimations impossible. Re-examination of samples after storage at -20°C for 6 months gave remarkably poor virus recoveries.

Method No. 59

70. Lue-Hing, C, B. T. Lynam, and J. R. Peterson. 1974. Digested Sludge Recycle to Land. Report No. 74-21, The Metropolitan Sanitation District of Greater Chicago.

Method Nos. 4, 8, 11, 30, and 52

71. Lue-Hing, C., S. J. Sedita, and K. C. Rao. 1977. Viral and Bacterial Levels Resulting From the Land Application of Digested Sludge. Report No. 77-21 Metropolitan Sanitary District of Greater Chicago. 44 pp.

Method Nos. 4, 8, 11, 30, and 52

72. Lue-Hing, C., D. Zenz, and J. Bertucci. 1973. Report on Inactivation of Viruses in Anaerobically Digesting Sewage Sludge. Metropolitan Sanitary District of Greater Chicago. 17 pp.

Methods Nos. 19, and 73

73. Malina, J., K.R. Ranganathan, B. Sagik, and B. Moore. 1975. Poliovirus Inactivation by Activated Sludge, J. Water Poll. Control Fed., 47(8): 2178-2183.

Tritium-labeled poliovirus (Mahoney) was added to batch activated sludge units to establish the mechanism of poliovirus inactivation and to investigate whether permanent inactivation takes place at the sludge-virus complex. The disposition of the virus particles in terms of their infectivity and the radiation count was followed with time over a period of 24 hr. The labeled virus was absorbed to the solids in the mixed liquor almost immediately, and 95 percent of the labeled virus was associated with the sludge solids after 10 min of contact. The level of radioactivity, measured as counts per minute, in the sludge essentially remained unchanged over the next 15 hr of aeration. The infectivity of the poliovirus (measured as plaque-forming units) in the supernatant decreased rapidly during this 15-hr aeration period, and the titer was 0.1 percent of the initial level. However, the infectivity of the polio virus in the sludge was lost at a much slower rate and, after the 15-hr aeration period, the titer was 20 percent of the initial level. The recovery of the radioactivity from the sludge solids was essentially 100 percent, and, at the same time, the recovery of infectivity as measured by plaque-forming units was about 20 percent. However, only the kinetics of inactivation were established, and the question of the mechanism of inactivation still must be answered.

Method No. 71

74. McKinney, R.E., H.E. Langley, and H.D. Tomlinson. 1958. Survival of Salmonella typhosa During Anaerobic Digestion. Sewage and Indust. Wastes, 30(12): 1469-1477.

The trend to shorter retention periods for sludge digestion and the lack of data on survival of enteric pathogens during digestion prompted this investigation. Spot-plate techniques using eosin-methylene-blue agar for Escherichia coli and Aerobacter aerogenes, and bismuth-sulfite agar for Salmonella typhosa were found to be satisfactory for enumeration of surviving enteric organisms. Normal raw sludges contained insufficient initial numbers and supplemental innoculations were therefore made. Relative death rates were highest in digesters with greater retention time, representative die-off rates for S. typhosa were 1.1 and 0.8 at 20 days and 6 days retention, respectively, when compared with initial daily counts of 450 thousand per ml.

Method No. 34

75. Metropolitan Sanitary District of Greater Chicago. 1979. Viral and Bacterial Levels Resulting from the Land Application of Digested Sludge. EPA 600/1-79-015, U.S. Environ. Protection Agency. 68 pp.

Method No. 52

76. Millner, P.D., P.B. Marsh, R.B. Snowden, and J.F. Parr. 1977. Occurrence of Aspergillus fumigatus During Composting of Sewage Sludge. Appl. and Environ. Microbiol., 34(6): 765-772.

Aspergillus fumigatus, a medically important fungal opportunist and respiratory allergen, was isolated from woodchips and sewage sludge used

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in the production of compost at the U.S. Department of Agriculture's composting research facility in Beltsville, Md. It was also regularly isolated as a dominant fungus during forced aeration composting and after 30 days in an unaerated stationary curing pile; in both cases, the fungus was found in pile zones with temperatures less than 60°C. Compost stored outdoors in stationary unaerated piles from 1 to 4 months after screening out of woodchips contained easily detectable amounts of A. fumigatus in the exterior pile zones (0- to 25-cm depths). Semiquantitative studies of the airspora at the composting site revealed that A. fumigatus constituted 75% of the total viable mycoflora captured. At locations 320 m to 8 km from the compost site, the fungus constituted only 2% of the total viable mycoflora in the air. Of 21 samples of commercially available potting soil, one had levels of A. fumigatus nearly equivalent to those of 1-month-old storage compost; 15 others had lower but detectable levels.

Method No. 50

77. Molina, J.A.E., O.C. Braids, and T.D. Hinesly. 1969. Bactericidal Properties of Digested Sludge Towards Fecal Coliforms, Abstract: Salmonella typhosa Tryptophan Deficiency, Prevention of Infection Spread. Bact. Proc., 69: 17.

Dangers of infection from fields irrigated with anaerobically fermented sewage sludge will be controlled by the persistence of pathogens in the digested sludge. The behavior of fecal coliforms in the sludge, as determined by the high temperature membrane filter technique, was investigated. Fecal coliforms, aerobically grown in a lactose broth and transferred to the sludge, died-off more rapidly if obtained from stock cultures than if freshly isolated from the sludge. This resistance of sludge isolates disappeared after several transfers in a lactose broth. The bactericidal properties were localized in the liquid phase of the sludge, but no antibiotics were detected by the diffusion agar method. The boiling of the sludge destroyed the bactericidal properties; yet, autoclaving did not. The lack of oxygen and the low oxido-reduction potential of the sludge were not solely responsible for the killing. Reversal of the bactericidal action was obtained by the addition to the sludge of high concentrations (5 gm/liter) of tryptone (Difco). At the same concentrations, tryptophane and tyrosine reduced the death rate slightly. Lactose or yeast extract, or both, had no effect. The disappearance of Salmonella typhosa in the sludge has been attributed to a nutritional deficiency in tryptophane. Another mechanism seems operative in the killing of nonadapted fecal coliforms.

Method: Not specified

78. Moore, B., B. Sagik, and J. Malina. 1975. Viral Association with Suspended Solids. Water Res. 9: 197-203.

Abstract laboratory models and field sewage samples were used to study viral association with particulates in suspension poliovirus and coliphages T2, T7, and 12 were tested for their adsorption to and elution from both organic and inorganic suspended solids at a variety of pH values and in both the presence and absence of a divalent cation. The results indicate that no one of the viruses tested can be used to describe a general pattern of adsorption. T2 and 12 readily associated with the clays used in the presence of divalent cation, while T7 and poliovirus absorbed equally well to both organic and inorganic particulates. All viruses varied in the degree to which they associated with naturally-occurring suspended solids in primary and final sewage effluents; maximum association occurred at the pH extremes of 4.0 and 10.0 for all systems. Regardless of the conditions used to offset adsorption, all viruses tested, with the exception of 12, were infective by plaque assay in then adsorbed form. These data suggest that no one coliphage system can acceptably model the behavior of poliovirus in laboratory or field conditions. Furthermore, the results reaffirm and broaden the findings that viruses associated with suspended particulates are infective. Therefore any system used to monitor virus levels in the environment must adequately account not only for free virus, but also for those which are solids-associated.

Method No. 70

79. Moore, B., B. Sagik, and C. Sorber. 1976. An Assessment of Potential Health Risks Associated with Land Disposal of Residual Sludges. In: Proceedings of the Third National Conference on Sludge Management, Disposal, and Utilization, Miami Beach, Fla., Dec. 14-16, 1976. pp. 108-112.

Method: Not specified

80. Moore, B.E., B.P. Sagik, and C.A. Sorber. 1978. Land Application of Sludges: Minimizing the Impact of Viruses on Water Resources. In: Pro. of Conf. on Risk Assessment and Health Effects of Land Application of Municipal Wastewater and Sludges, Sagik and Sorber, eds., San Antonio, Tex. pp. 154-167.

Sludges, particularly activated sludges, sequester the major portion of viruses that can be recovered from raw wastewaters. Anaerobic digestion reduces the level of viruses which can be recovered from secondary sludges. Because of short circuiting and overloading, the viral reductions achieved are significantly less in practice than those obtained in laboratory models. Even digested sludges carry infectious viruses into the soil where their survival is a function of regional or local climatic conditions. Such survival can be quite extended in the land-applied sludge and soil systems. Low temperatures are particularly conducive to prolonged viral survival. The transport of viruses in soil systems can be anticipated where precipitation or nonwastewater irrigation is sufficient to effect changes in the ionic milieu of the aerated zone.

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Sludge-occluded poliovirus can be recovered from soil column percolates at levels ranging from 1% to about 6% of the recovery obtained with viruses applied in effluent. The risk associated with the potential viral contamination of groundwater or surface waters from sludge injection practices can be minimized by effective treatment of sludges prior to injection or land application. Anaerobic digestion can be such a treatment provided that reasonably long detention times and high temperatures are employed and that short circuiting is eliminated by proper design and operation. If these conditions cannot be achieved, other forms of sludge treatment may be warranted.

Method No. 54

81. Morgan, M., and F. McDonald. 1969. Test Show MB Tuberculosis Doesn't Survive Composting. J. Environ. Health, 32(1): 101-108.

Method No. 40

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82. Morris, M.E., J.S. Sivinski, J.R. Brandon, K.S. Neuhauser, and R.L. Ward. 1979. A Summary of Recent Developments in the Sludge Irradiation Program at Sandia Laboratories. SAND79-0629, Sandia Laboratories, Albuquerque, NM. 57 pp.

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Method: Not specified

83. Muller, G. 1959. Tubercle Bacteria in Sludge From Mechanical-Biological Sewage Treatment Plants. Stadtehygiene, 10: 96-97.

After a summary of the findings of other investigators on the effect of sewage treatment on tubercle bacteria, the author describes the results of examination of 590 samples of crude sewage and sludges from a plant in which sewage was treated by sedimentation, biological filtration, and the activated-sludge process, and sludge was digested in heated and unheated tanks. 90 percent of the samples of crude sewage were positive; sludge from lightly loaded percolating filters was also positive in 90 percent of the samples; 29-50 percent of samples of sludge from high-rate filters and of activated sludge were positive; 50 percent of samples of sludge from unheated digestion tanks were positive but no bacteria were found in sludge from heated digestion tanks; sludge dried for about 14 days gave 71 percent positive samples and the percentage after about 30 days was 44. The importance of heat treatment if sewage or sludge is to be used for agricultural purposes is pointed out.

Method No. 37

84. Nath, M.W., and J.C. Johnson. 1979. Quantitative Enumeration and Evaporation- Induced Inactivation of Enteric Viruses in Wastewater

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Sludge. Virginia Water Resources Research Center, Virginia Polytechnique Institute. 51 pp.

This study examined a) different methodologies used to isolate, concentrate, and enumerate enteric viruses in primary sludge; b) evaporation of primary sludge as a virucidal process.

Viral enumeration by plaque assay in methylcellulose medium was found to be dependent on host cell type. Poliovirus type 2, strain W-2 [ATCC-VR30-(Po-2)] was most efficiently titered on RD cells relative to BGM, VERO, WI-38, or LLC-MK_2 cells. Coxsackievirus type B-2, strain Ohio-1 [ATCC-VR29 (Cox B-2)] was most efficiently plaqued on BGM cells. Cox B-2 infections of RD cells resulted in diffuse, incomplete cytopathic effects not easily enumerated. The Anatid herpesvirus, Holland strain [ATCC-VR684 (AHV)], a virus known to be transmitted by water, was infectious only for cells of avian origin.

More than 85 percent of a highly purified [^{14}C]-Po-2 stock was found to be rapidly adsorbed to the solids fraction of primary sludge obtained from the James River Wastewater Treatment Plant during an eight-month sampling period. A 40-fold dilution of primary sludge solids resulted in less than a 2-fold reduction in bound Po-2. Seldom were indigenous viruses in centrifugation clarified supernatant fractions of primary sludge detected without concentration.

Sodium dodecyl sulfate (SDS) was found to be most effective in comparative studies with five other surfactants, fetal bovine serum, and NaCl in desorbing Po-2 and Cox B-2 from sludge solids. Infectivity recoveries of greater than 95 percent were obtained using 0.2 percent SDS at neutral pH and 22°C to desorb Cox B-2 from solids. The efficiency of Po-2 extraction was equally high. The SDS extraction procedure was found to be dependent on the percent of sludge solids for any particular sample and varied (5.3 to 89.5%) for samples collected during the study period.

Higher yields of Po-2 were obtained from seeded sludges in the absence of surfactants using a multiple extraction-concentration procedure. Virus concentration was by ultracentrifugation. Using this multiple extraction-concentration procedure the average yield for all Po-2 seeded sludges was 87.4 percent. Considering the 20-fold concentration achieved, enteric virus amounts in the range of 50 to 100 pfv/l would likely be detected provided the use of susceptible cells.

Indigenous virus concentrations in primary sludge were found to vary from less than 50 to 100 pfv to 1.9×10^4 pfv/l as assayed on VERO cells and 4.6×10^4 to 3.4×10^7 coliphage/l during a 7-month sampling period. For any individual sample the coliphage to eukaryotic virus ratio varied from between $\cong$13 and 28,000 to 1.

The process of evaporation was found to be virucidal for Po-2. At 22.5°C the rate of inactivation for Po-2 in water was approximately 1

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log/day. This rate did not appreciably change for Po-2 in primary sludge under the same conditions. At about 55 percent solids a breakpoint in the rate of inactivation resulted in the loss of all detectible infectivity (>3.0 log) within 1.5 hr. The same response and breakpoint were found for Po-2 in sludge dewatered at 37°C. The results were interpreted in terms of a concentration-induced activation of a virucidal component of primary sludge.

Method No. 72

85. Newton, W.L., H.J. Bennett, and W.B. Figgat. 1949. Observations on the Effects of Various Sewage Treatment Processes upon Eggs of *Taenia saginata*. Am. J. Hygiene, 49: 166-175.

Method No. 86

86. Newton, W.L., W.B. Figgat, and S.B. Weibel. 1948. The Effects of Sewage Treatment Processes Upon Ova and Miracidia of *Schistosoma japonicum*. Part II. Sewage Works J. 20(4): 657-664.

Method: Not specified

9-1/8"

87. Nielsen, A.L., and B. Lydholm. 1980. Methods for the Isolation of Virus from Raw and Digested Wastewater Sludge. Water Res. 14(2): 175-178.

Four different quantitative methods for detection of viruses in wastewater sludges were tested on raw and digested sludge. Acidic precipitation followed by elution with beef extract and alum precipitation followed by elution with tris buffer seemed equally good. Elution with beef extract also gave a good recovery and was especially usable for sludge with high amounts of total suspended solids. Direct inoculation of sludge into cell cultures often had a harmful effect on the cells. All the samples of raw sludge contained viruses and the concentration was higher than in the digested sludge. The types isolated were coxsackievirus B2 and B5, poliovirus 1, 2 and 3, and adenovirus 1 and 2. In one sample adenovirus type 2 was isolated one day after land application and in another coxsackierivus B5 was isolated 4 months after.

Method No. 56

88. Noland, R.F., J.D. Edwards, and M. Kipp. 1978. Full Scale Demonstration of Lime Stabilization. EPA-600/2-78-171. U.S. Environ. Protection Agency, Cincinnati, Oh. 75 pp.

Methods Nos. 1, 4, 6, 8, 11, and 30

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89. Palfi, A. 1972. Survival of Enteroviruses during Anaerobic Sludge Digestion. In: Advances in Water Pollution Research, Proc. of the 6th Intl. Conf., S.H. Jenkins, ed., Jerusalem, Israel. pp. 99-107.

Method No. 53

90. Passman, F. 1978. Fate and Occurrence of Pathogens. In: Draft Position Papers for the Workshop on the Health and Legal Implications of Sewage Sludge Composting (Energy Resources Co., Inc.), Cambridge, MA. pp. 3-1 - 3-30.

Method: Not specified

91. Rawn, A.M., and E.J. Candell. 1950. Some Effects of Anaerobic Digestion on Sewage Sludge. Trans. ASCE. 115: 181-193.

Method No. 1

6-1/2"

92. Reeves, J.B. 1959. Sanitary Aspects of Composted Sewage Sludge and Sawdust. Sewage and Indust. Wastes. 31(5): 557-564.

Method Nos. 8, and 21

93. Reimers, R.S., M.D. Little, A.J. Englande, D.B. Leftwich, D.D. Bowman, and R.F. Wilkinson. 1980. Parasites in Southern Sludges and Disinfection by Standard Sludge Treatment. In preparation, EPA Grant No. R-805107-01, U. S. Environmental Protection Agency, Cincinnati, Ohio. 190 pp.

This study (1) assessed the presence and densities of parasites in municipal wastewater sludges in the southern United States; (2) investigated the inactivation of parasites by lime stabilization of sewage sludges seeded with selected intestinal parasites; (3) measured a mass balance of parasites through a WWTP; (4) assessed standard sludge treatment practices from laboratory and field data for the control of parasites.

Sludge samples collected during each of the four seasons from 27 municipal wastewater plants located in Alabama, Florida, Mississippi, Louisiana and Texas were examined for the presence and densities of resistant stages of human and animal parasites. Viable eggs of Ascaris and Toxocara were recovered at least once from every plant and viable eggs of T. vulpis and T. trichiura were recovered at least once from 26 and 15 plants, respectively. Viable eggs of at least 10 other helminths and cysts of a few protozoa were also found in fewer numbers and less frequently. Certain drying bed conditions such as previous sludge stabilization, high temperature and low moisture content appear to inactivate parasite eggs consistently between 60% and 5% sludge moisture

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content. Results of the mass balance study indicated that the clarifier processes tended to concentrate and equalize parasite concentrations; the clarifier overflow rate affected removal efficiency of parasites. Laboratory studies verified results of previous investigations indicating that destruction of resistant parasite eggs is primarily due to temperature and not to a specific digestion process. The application of lime to primary, aerobic-digested, and anaerobic-digested sludge was found to be effective with >80% reduction of *Ascaris* viability in five days following aerobic digestion at a lime $(Ca(OH)_2)$ dosage of about 1g/g dry weight of sludge solids. Laboratory experiments also showed that at certain combinations of ultrasonic frequency intensity, and exposure time *Toxocara* eggs could be destroyed, but that the same ultrasonic conditions did not affect *Ascaris* eggs. This study also demonstrated that eggs taken from the uteri of live *Ascaris* females are not suitable for use as an indicator parasite in laboratory inactivation studies.

Method No. 96

94. Reyes, W., C. Kruce, and M. Batson. 1963. The Effect of Aerobic and Anaerobic Digestion on Eggs of *Ascaris lumbriocoides* var *suum* in Nightsoil. Am. J. Tropical Med. Hygiene, 12: 46-55.

Ascaris ova which fail to develop during sludge treatment may be viable and may recover if incubated under more favorable conditions. Ova which failed to develop were removed from the digesters and placed on moist soil at 30°C for 16 days. Suspension in water with aeration worked as well. When nightsoil which is 3 percent solids was inoculated with 15,000 to 30,000 ova per ml and anaerobically digested, there was a marked retardation in development but 15 percent recovered when the digester temperature was 30°C. At 38°C, there was no recovery; temperatures above 40°C interfere with ova hatching. Between 25 and 35°C, there was some destruction of ova not caused by heat. In the anaerobic system, this was attributed to O_2 starvation. In the aerobic system, to spontaneous hatching. At lower temperatures both systems preserve *Ascaris*. 38°C is fatal during anaerobic digestion; 45°C during aerobic digestion. Heating nightsoil to 55°C for 20 min kills *Ascaris*.

Method No. 86

95. Sacramento Area Consultants. 1979. Sewage Sludge Management Program. Vol. 6: Microbiological and Virus Studies.

Method Nos. 8, 11, 29, and 95

96. Sagik, B.P., and C.A. Sorber. 1978. Risk Assessment and Health Effects of Land Application of Municipal Wastewater and Sludges. Proc. of Conf., San Antonio, Tex., 1977. NSF/RA-780106, Nat. Sci. Foundation, Washington, D.C., Pub. 344 pp.

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These proceedings are intended as a record of the conference on risk assessment and health effects of land application of municipal wastewater and sludges. The topic on agents of health significance covered health hazards associated with wastewater effluents and sludges; chemical agents of health significance in municipal wastewater effluents and sludges; and conventional methods of epidemiology appropriate for risk assessment of virus contamination of water. The examination of the impact on the food chain included discussions on opportunities for virus transport; land application of sewage wastes; problems in proving that parasites pass to people from foods produced with refuse; the impact of metals present in municipal sludges; and the potential health hazard and risk assessment of toxic organic chemicals in wastewater and sludge. The impact on water resources is discussed in terms of minimizing the impact of viruses on water resources; minimizing the virological impact on water resources from spray irrigation of municipal wastewater; potential impact on water resources of bacterial pathogens; obligate parasites; limitations for land disposal of wastewater and sludges; and viruses in the environment. Pathogenic aerosols are discussed in terms of aerosol monitoring for microbial organisms and a model for predicting pathogen concentrations in wastewater aerosols. Factors affecting risk assessments are evaluated in terms of wastewater unit process efficiency; extrapolation models for risk assessment; and public choice and the land application of municipal wastewaters and sludges.

Method: Not specified

97. Sanders, D.A., J.F. Malina, B.E. Moore, B.P. Sagik, and C.A. Sorber. 1979. Fate of Poliovirus During Anaerobic Digestion. J. Water Poll. Control Fed., 51(2): 333-343.

Method No. 67

98. Sattar, S.A., S. Ramia, and J.C.N. Westwood. 1976. Calcium Hydroxide (Lime) and the Elimination of Human Pathogenic Viruses from Sewage: Studies with Experimentally-Contaminated (Poliovirus type 1, Sabin) and Pilot Plant Studies. Canadian J. Public Health, 67(3): 221-226.

Method No. 68

99. Sattar, S.A., and J.C.N. Westwood. 1976. Comparison of Four Eluents in the Recovery of Indigenous Viruses from Raw Sewage. Canadian J. of Microbiol., 22(10): 1586-1589.

The efficiency of 3% casein hydrolysate (CH), 3% lactalbumin hydrolysate (LH), 3% beef extract (BE), and 10% fetal calf serum (FCS) was compared for the recovery of viruses from raw sludge. CH and LH proved to be inefficient and were eliminated from the study after initial testing. In tests with 20 different samples of raw sludge, beef extract eluted

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virus in 15 (75%) and FCS revealed virus in 19 (95%) of the samples using BS-C-1 cells. That different eluents were not eluting different viruses from the same sample was shown by the serologic and electron-microscopic examination of 43% (18/42) of the isolates. The identified viruses included memebers of the entero (coxsackie B, and polio) and reo-virus groups.

Method No. 58

100. Sattar, S.Y., and J.C.N. Westwood. 1979. Recovery of Viruses from Field Samples of Raw, Digested and Lagoon-Dried Sludges. Bulletin World Health Org., 57(1): 105-108.

In a 22-month study, viruses were detected in 84% (62/74) of raw, 53% (19/36) of anaerobically digested, and 39% (11/28) of lagoon-dried sludge samples. Lagoon sludge contained detectable viruses (reovirus and enterovirus groups) even after 8 months of retention. Because of such prolonged virus survival in sludge, care must be taken in its disposal or utilization.

Method No. 62

101. Schatzle, M. 1969. Investigations on the Effect of Sewage Sludge from Oxidation Channels on the Viability of Worm Eggs. Z. Wass. Abwass. Forsch., 2(4): 147-150.

Method No. 80

102. Shook, W.M., R.B. Czarnecki, and R.C. Perriello. 1962. Bacteriology of Vacuum Filtered Raw Sludge. Water and Sewage Works, 109(4): 152-155.

Study was made of relatively new method of sewage treatment and disposal, i.e., dewatering of fresh undigested sewage solids by vacuum filtration; purpose was to determine degree of coliform elimination during treatment and storage of this undigested, dewatered sludge and to discover possible extent of gram-negative enteric pathogen survival; consistent pattern in cell population was noted among samples of stored sludge; recommendations made. 21 refs.

Method Nos. 1, and 21

103. Silverman, P.H., and K.J. Guiver. 1960. Survival of Eggs of *Taenia saginata* (The Human Beef Tapeworm) After Mesophilic Anaerobic Digestion. J. and Proc. Inst. Sewage Purif., Part 3, pp. 345-347.

Method No. 89

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104. Sivinski, H.D. 1975. Progress Report, Waste Resources Utilization Program, Waste Management and Environmental Programs Department 5440, Sandia Laboratories, Albuquerque, New Mexico. SAND75-0436. 35 pp.

Method: Not specified

105. Sivinski, H., J. Brandon, M. Morris, K. Neuhauser, and R. Ward. 1977. Recent Developments in the Sandia Laboratories Sewage Sludge Irradiation Program. In: Sandia Lab. Report, SAND77-1411, November, 1977.

Pathogen reduction studies have shown that a 1 Mrad treatment (or less at elevated temperatures) is very effective in eliminating pathogenic bacteria and viable parasite ova in liquid sludges. Heat is effective in reducing levels not only of pathogenic bacteria and *Ascaris* ova, but viruses as well. Ammonia has been shown to be virucidal to poliovirus and several other enteric viruses. Dried, irradiated undigested sludge has been shown to be of significant nutritional value when used as a feed supplement for sheep and cattle, as well as in agronomic uses. No significant harmful effects have been noted in the feeding program thus far.

Method: Not specified

106. Steer, A.G., J.H. Nell, and S.G. Weichers. 1974. A Modification Allen and Ridley Technique for the Recovery of *Ascaris lumbricoides* Ova from Municipal Compost. Water Res., 8: 851-853.

The Allen and Ridley method for the isolation of parasitic ova from sewage sludge was modified by the addition on an anionic detergent to a larger test sample. This method was equally efficient for counting *Ascaris lumbricoides* ova in raw sludge and in various mixtures of sludge and compost. The modified method yielded considerably higher counts for all samples studied than the original method.

Method No. 78

107. Stern, G., and J.B. Farrell. 1977. Sludge Disinfection Techniques. In: Proc. of Nat. Conf. on Composting of Municipal Residues and Sludges, Washington, D.C., Information Transfer, Inc., Rockville, MD. pp. 142-148.

Method: Not specified

108. Stroh, R. 1961. Possibility for the Destruction of Parasites in Heated and Unheated Digestion Tanks. Muenchner Beitr. Abwasser, Fish. Flussbiol. (Ger.), 8: 95-103.

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The problem of complete destruction of zoological parasites and pathogenic bacteria during sludge digestion is discussed. Previous experiences showed that viable eggs do not develop after 3 months' detention in an unheated tank. Studies on the development of eggs of Ascaris lumbricoides placed in heated digestion tanks in containers similar to that used by Brunner, G., showed that the eggs were still viable after 15 days, but after 2 months no viable eggs were found. From the latest research, it is claimed that total destruction can be achieved by reheating the sludge for 6 minutes at 70°C after retention for 65 days in the digestion chamber. Liebmann and Offhaus achieved total destruction of parasitic eggs by sterilization of fresh sludge at 100°- 120°C for 15-20 min.

Method No. 82

109. Subrahmanyan, T.P. 1977a. Persistence of Enteroviruses in Sewage Sludge. Bull. World Health Org., 55(4): 431-434.

Sewage from residential areas often contains viruses pathogenic for man and significant amounts are probably associated with solids in sewage sludge. Information on the survival of viruses in sewage sludge is necessary in order to develop guidelines for recycling programmes that involve spreading the sludge on land. In the present study, a number of enteroviruses were added to sewage sludge and the artificially contaminated sludges were tested for viruses at intervals over a 12-week period. Most of the viruses survived for many weeks at room temperature. It is clear that sewage sludge destined for land application should be adequately treated for virus inactivation. In interpreting these results, it should be borne in mind that the survival of hepatitis A virus might be similar. Recent reports about the reappearance of poliomyelitis in regions with immunization programmes should also be taken into consideration.

Method No. 76

110. Subrahmanyan, T.P. 1977b. Examination of Sewage And Sewage Sludge for Enteroviruses. Vol. II. Res. Rept. No. 52, Canada-Ontario Agreement on Great Lakes Water Quality. 33 pp.

This report deals with the first phase of the program at the Enterovirus Laboratory, Ontario Ministry of Health, Laboratory Services Branch, relating to spreading urban sewage sludge on rural land in Ontario.

Results include virus surveillance studies at five plants using different phosphate removal techniques, and related laboratory studies. The plants involved were the North Toronto pollution control plant, and the treatment plants at Lakeview, Newmarket, Point Edward and Sarnia. Plants at Brampton, Burlington and the other Metro Toronto plants were included for comparison.

During April 1973 - December 1975, a total of 1,317 specimens, including 642 sewage specimens, 540 raw and digested sludge specimens, and 135 specimens of water were investigated. Out of the 444 specimens tested in 1973, 40% of the sewage specimens (48/124) contained virus, although the collection period included several cold months during which enteroviruses were not detected in the community. Virus isolation rates in 1974 and 1975 were lower, which parallel the lower isolation rates from clinical cases as well. The virus isolates from sewage included several poliovirus isolates. Preliminary results indicate that at least one strain is definitely not derived from vaccine; a few others are intermediate in character, indicating that they too could be of nonvaccine origin. All the nonpolio enteroviruses found in this study are well-known human pathogens and, therefore, it is important to ensure that they are inactivated before sludge is spread on soil. One important shortcoming of this and similar studies is the present nonavailability of a suitable test for the detection of hepatitis and gastroenteritis viruses. Until this is resolved, the real risk of viral contamination cannot be accurately assessed. Thus, our conclusions have to be considered provisional.

In the present study several enteroviruses persisted in sludge many weeks. This also agrees with limited experience in the field. Sludge utilization programs should take into account the possible presence of pathogenic viruses in a sludge and the potential for an extended health hazard. The presence of the more easily inactivated reoviruses in sludge suggests that the more virulent enteric viruses were not found only because they were not circulating widely in the community.

Method No. 76

111. Taffel, W. 1978. Health Risk Assessment. In: Draft Position Papers for the Workshop on the Health and Legal Implications of Sewage Sludge Composting. Energy Resources Co., Inc., Cambridge, MA.

Method: Not specified

112. Taylor, J.M., E. Epstein, and W.D. Burge et al. 1978. Chemical and Biological Phenomena Observed with Sewage Sludges in Simulated Soil Trenches. J. Environ. Qual., 7 (4): 477-482.

Limed and unlimed raw and digested sludges were placed in simulated trenches in soil profile boxes with sown corn for the 160 day growth period. The following conditions were observed in the raw sludge: severely restricted root penetration; prevailing anaerobic periods; 45% methane (CH4) level; and 25% carbon dioxide (CO_2) level. Roots penetrated and grew throughout digested sludge which had <3%-CH4, 21%-CO_2, and a higher nitrate nitrogen than raw sludge. Zinc and Cu did not translocate into the surrounding soil. The uptake of these metals into corn leaves was relatively low, reaching 131 µg/g Zn and 5.9 µg/g Cu as

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compared with 79 μg/g Zn and 3.8 μg/g Cu in the controls. Low levels of fecal coliforms survived in the sludge and correlated negatively with ammonia-N concentrations, suggesting that ammonia is important in reducing human pathogens. No coliforms were found in the surrounding soil.

Method Nos. 1, and 6

113. Theis, J.H., V. Bolton, and D.R. Storm. 1978. Helminth Ova in Soil and Sludge from Twelve U.S. Urban Areas. J. Water Poll. Control Fed., 50 (11): 2485-2493.

The prevalence of parasitic helminth ova in sludge, soil upon which sludge had been deposited, and irrigation return flow from areas which had received sludge was determined microscopically. Standard screening and flotation techniques were used in processing the sludge, soil, and water. The prevalence of ova varied from one geographic area to another as did the particular helminths found. *Ascaris lumbricoides* were the ova most frequently recovered, in an average of 25.8% of all sludge samples, 7.4% of all water samples, and 1.6% of all soil samples examined over the 34-mo. period. *Toxocara* sp. was the next most prevalent ovum found, indicating the extent to which pet animal fecal material finds its way into the sewer system of urban areas. Viability testing, following a special composting experiment during which temperatures of 63°C were achieved, showed that all seeded *Ascaris* ova failed to embryonate.

Method No. 93

114. Trubnick, E.H., and P.K. Mueller. 1958. Sludge Dewatering Practice. Sewage and Indust. Wastes, 30 (11): 1364-1378.

Method: Not specified

115. Veerannan, K.M. 1977. Some Experimental Evidence on the Viability of *Ascaris lumbricoides* Ova. Current Sci., 46 (11): 386-387.

Method No. 83

116. Wang, M.H., L.K. Wang, G.C. Peery, III, and C.M. Cheung. 1978. General Theory of Chemical Disinfection and Sterilization of Sludge; Part 3. Water and Sewage Works, 125 (9): 99-104.

The efficiency of chemical disinfection in water is dependent on the nature of organisms and their concentration and condition; on the nature, concentration, distribution, and chemical form of the disinfectant; on the condition of the water to be treated; on the temperature of the water; and on the time of contact. Most organisms are more easily killed in acid or alkaline solution than in a neutral environment. Lime addition raises the pH to stabilize wet sludge and destroy pathogens; lime sludge dewaters well without odor problems. The key to Cl disinfection efficiency is the concentration of undissociated hypochlorous acid, which is higher at lower pH. Chlorine dioxide does not have the taste and odor problems associated with Cl disinfection, is not affected by the presence of organic materials in the water, and is effective at high pH. Ozone also causes no taste or odor problems, poses no potential hazard to aquatic life or the general population, and may be superior to Cl as a virucide, but has higher capital and operating costs. Iodine and Br2 have also seen limited use as disinfectants. Silver ions are used for disinfection in Europe. Quaternary ammonium compounds are equally effective against gram positive and gram negative organisms, with the bactericidal efficiency dependent on the number and nature of the alkyl groups. The combination of an insoluble quaternary ammonium resin with tri-iodide ions has strong antibacterial properties. The disinfection efficiency of potassium permanganate is not as good as that of Cl.

Method: Not specified

117. Ward, R.L. 1977. A Program to Study the Inactivation of Enteric Viruses in Wastewater Sludge. In: ERDA Symposium Series 44, Baltimore, MD. pp. 40-53.

Sludge contains a natural virucidal agent for enteroviruses which is the uncharged form of ammonia. This agent also causes the rate of heat inactivation of these viruses to be accelerated. Sludge protects poliovirus against inactivation by both heat and radiation, but much of this protection can be eliminated in low concentrations of sludge when the two are applied simultaneously.

Method: Not specified

118. Ward, R.L. 1978. Pathological Aspects of Inactivation of Enteric Viruses in Dewatered Wastewater Sludge. In: Proc. of Nat. Conf. on Design of Municipal Sludge Compost Facilities, Chicago, Ill. pp. 42-47.

Method No. 74

119. Ward, R., and C. Ashley. 1976. Inactivation of Poliovirus in Digested Sludge. Appl. and Environ. Microbiol., 31(6): 921-930.

The effect of anaerobically digested sludge on poliovirus during incubation at temperatures between 28 and 4°C was studied. Although virus was fully recoverable from sludge, its infectivity decreased in proportion to the time and temperature of incubation. The rate ranged from greater than 1 log per day at 28°C to about 1 log every 5 days at 4°C. The mechanism of inactivation was studied at the lower temperature where the sedimentation coefficients of most inactivated particles were not detectably modified. The ribonucleic acid (RNA) of these particles appeared to have been nicked and had an average sedimentation value about 70% that of RNA from infectious virus. Since the specific infectivity of RNA from particles recovered from sludge was directly proportional to that of the particles from which it was extracted, loss of infectivity was probably due to inactivation of RNA. Some breakdown was also found in the two largest viral proteins of inactivated particles. Thus, the mechanism of inactivation may be cleavage of viral proteins followed by nicking of encapsulated RNA. Because no virucidal activity was found in raw sludge, this component of digested sludge appears to be a product of the digestion process.

Method No. 74

120. Ward, R., C. Ashley, and R. Moseley. 1976. Heat Inactivation of Poliovirus in Wastewater Sludge. Appl. and Environ. Microbiol., 32(3): 339-346.

The effect of raw and anaerobically digested sludge on heat inactivation of poliovirus was investigated. Raw sludge was found to be very protective of poliovirus plaque-forming ability at all temperatures studied, but digested sludge had variable effects that were highly dependent upon the experimental conditions. In low concentrations and at relatively low inactivation temperatures, digested sludge is nearly as protective of poliovirus as raw sludge. However, at higher temperatures and concentrations, digested sludge caused a significant acceleration of poliovirus inactivation. The difference between the protective capability of raw and digested sludge is not due to loss of protective material, because this component is present in the solids of digested sludge as well as in those of raw sludge. Instead, the difference is due to a virucidal agent acquired during digestion. Addition of this agent to the solids of either raw or digested sludge reverses the protective potential of these solids during heat treatment of poliovirus.

Method No. 74

121. Ward, R., and C. Ashley. 1977a. Identification of the Virucidal Agent in Wastewater Sludge. Appl. and Environ. Microbiol., 33(4): 860-864.

Anaerobically digested sludge contains an agent that causes irreversible inactivation of poliovirus. It has now been shown that the agent responsible for this activity is ammonia. The effect of ammonia on poliovirus appears to be typical for picornaviruses, but reovirus, an enteric virus of another group, is quite resistant to this compound. Because ammonia is not virucidal in its charged state, it expresses

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significant activity only at pH values greater than 8. Therefore, increasing the pH of sludge should cause rapid inactivation of indigenous picornaviruses.

Method No. 74

122. Ward, R.L., and C.S. Ashley. 1977b. Inactivation of Enteric Viruses in Wastewater Sludge Through Dewatering by Evaporation. Appl. and Environ. Microbiol., 34(5): 564-570.

The effect of dewatering on the inactivation rates of enteric viruses in sludge was determined. For this study, water was evaporated from seeded raw sludge at 21°C, and the loss of viral plaque-forming units was measured. Initial results with poliovirus showed that recoverable infectivity gradually decreased with the loss of water until the solids content reached about 65%. When the solids content was increased from 65 to 83%, a further, more dramatic decrease in virus titer of greater than three orders of magnitude was observed. This loss of infectivity was due to irreversible inactivation of poliovirus because viral particles were found to have released their RNA molecules which were extensively degraded. Viral inactivation in these experiments may have been at least partially caused by the evaporation process itself because similar effects on poliovirus particles were observed in distilled water after only partial loss of water by evaporation. Coxsackievirus and reovirus were also found to be inactivated in sludge under comparable conditions, which suggests that dewatering by evaporation may be a feasible method of inactivating all enteric viruses in sludge.

Method No. 74

123. Ward, R.L., and C.S. Ashley. 1978a. Identification of Detergents as Components of Wastewater Sludge that Modify the Thermal Stability of Reovirus and Enterovirus. Appl. and Environ. Microbiol., 36(6): 889-897.

The agent in wastewater sludge previously shown to reduce the heat required to inactivate reovirus (R.L. Ward and C.S. Ashley, Appl. Environ. Microbiol. 34:681-688, 1977) was "separated" from other sludge components and analyzed by infrared spectroscopy. The infrared spectrum of this material was quite similar to the spectra of commercial anionic detergents, and subsequent analyses of the fractionated sludge samples revealed that anionic detergents in sludge were copurified with the virucidal activity. Further measurements on the virucidal activities of specific detergents revealed that ionic detergents reduce the heat required to inactivate reovirus, that cationic detergents are more active than anionic, and that nonionic detergents are inactive. Several detergents were also shown to protect poliovirus and other enteroviruses against inactivation by heat. These results indicate that ionic detergents are the major component in wastewater sludge that reduce the

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thermal stability of reovirus and, in addition, that detergents are able to protect enteroviruses against heat.

Method No. 74

124. Ward R.L., and C.S. Ashley. 1978b. Heat Inactivation of Enteric Viruses in Dewatered Wastewater Sludge. Appl. and Environ. Microbiol., 36(6): 898-905.

The effect of moisture content on the rates of heat inactivation of enteric viruses in wastewater sludge was determined. The protective effect of raw sludge on poliovirus previously observed (R.L. Ward, C.S. Ashley, and R.H. Moseley, Appl. Environ. Microbiol. 32:339-346, 1976) was found to be greatly enhanced in sludge dewatered by evaporation. Other enteroviruses responded in a similar fashion. This effect did not appear to be due merely to the state of dryness of the sludge samples because in humus-deficient soil, a relatively inert material, the rate of poliovirus inactivation by heat was not significantly altered through dewatering. Instead, this effect appeared to have been caused by protective substances in the sludge, such as detergents, which are concentrated through dewatering. As reported previously (R.L. Ward and C.S. Ashley, Appl. Environ. Microbiol. 34:681-688, 1977; R.L. Ward and C.S. Ashley, Appl. Environ. Microbiol. 36:889-897, 1978) raw sludge is not protective of reovirus, but, instead, the ionic detergents in sludge cause the rate of heat inactivation of this virus to be accelerated. Dewatering of sludge, however, was found to partially reverse this virucidal effect. Evidence is presented indicating that this reversal is caused by an unidentified protective substance in sludge also concentrated through dewatering. Finally, it was shown that the effects of raw sludge on heat inactivation of poliovirus and reovirus are greatly reduced by composting, a result that correlated with the degradation of detergents.

Method No. 74

125. Ward, R.L. and C.S. Ashley. 1978c. Methods to Inactivate Enteric Viruses in Wastewater Sludge. SAND 77-1498, Sandia Labs., Albuquerque, NM. 25 pp.

Sewage sludge contains an agent that causes inactivation of poliovirus at temperatures at least as low as 4°C. This agent has been identified as ammonia. The mechanism by which ammonia inactivates poliovirus is to stimulate cleavage of viral RNA within the intact virion. Sludge also contains material that is protective of poliovirus against heat inactivation, but its effect can be reversed by ammonia. The protective substance may be the same component of sludge that greatly reduces the temperature required to inactivate reovirus. This substance apparently becomes concentrated during the drying of sludge but was not found in composted sludge, which suggests that it is destroyed during composting.

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Method No. 74

126. Ward, R.L., and C.S. Ashley. 1979. Effects of Wastewater Sludge and Its Detergents on the Stability of Rotavirus. SAND 79-2159J, Sandia Labs., Albuquerque, NM. 23 pp.

Method No. 74

127. Ward, R.L., and J.R. Brandon. 1977. Effect of Heat on Pathogenic Organisms Found in Wastewater Sludge. CONF-770838-1, Conf. on Composting of Municipal Residues and Sludges, Silver Spring, Md. pp. 122-127.

To overcome the biological risk associated with sewage sludge utilization as a soil conditioner, fertilizer, or food-supplement for animals low-cost methods are being sought which can be used to efficiently inactivate pathogenic organisms in sludge. One possible method that is being carefully examined at Sandia Laboratories is treatment with large doses of ionizing radiation. The economic feasibility of this procedure depends on the availability of large amounts of cesium-137 found in nuclear wastes. Initial experiments suggest that pathogenic bacteria and parasites can be effectively inactivated in sludge by exposure to this gamma source. However, viruses are intrinsically radiation resistant and very large doses of ionizing radiation are required for their inactivation in sludge. Another means of inactivating pathogens in sludge is with heat. Because viruses are thought to be particularly labile at high temperatures, heat treatment should be especially effective against this class of pathogens. Therefore, this treatment is being considered as a possible add-on to radiation treatment and, accordingly, studies on heat inactivation of enteric viruses, bacteria, and parasites are under way. Some of the findings are reported.

Method: Not specified

128. Wellings, F.M., A.L. Lewis, and C.W. Mountain. 1976. Demonstration of Solids Associated Virus in Wastewater and Sludge. Appl. and Environ. Microbiol., 31(3): 354-358.

Data presented demonstrate the relatively high multiplicity of solids-associated virus in field samples, i.e., wastewater, sludge, and soils. Influent, effluent, and chlorinated effluent samples showed 16.1 to 100% of the total virus demonstrated in samples to be solids associated. Three techniques for freeing solids-associated virus are described and compared. Using sonication of solids and polyethylene glycol concentration, virus was demonstrated in fully digested sludge (60 days at 34°C), sand at the site of a sewer leak, and dried sludge cake and mud 900 m downstream from a sewage disposal site. These data emphasize the inadequacy of virus concentration techniques that do not include the processing of solids. In situ elution failed to free solids-associated virus.

Method No. 52

129. Wiley, J.S. 1962. Pathogen Survival in Composting Municipal Wastes. J. Water Poll. Control Fed., 34 (1): 80-89.

After 43 hours of composting, *Salmonella newport*, poliovirus 1, *Ascaris lumbricoides* ova, and *Candida albicans* that had been added to raw sewage sludge were no longer detectable. The poliovirus 1 was most sensitive, requiring one hour for complete inactivation, and *C. albicans* was the most resistant, requiring more than 28 hours of composting for complete inactivation. The data from this study indicated that aerobic composting of sewage sludge destroys the pathogens when a temperature of 60 to 70°C is maintained for three days.

Method: Not specified

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Studies were conducted to assess the effectiveness of an aerobic composter in destroying pathogens that may possibly be present in raw sewage sludge. Experiments conducted in this study were designed to determine whether or not selected indicator organisms (i.e., *Salmonella newport*, poliovirus type 1, *Ascaris lumbricoides* ova, and *Candida albicans*) could survive the composting process. The results of the assay showed that after 43 hr of composting, no viable indicator organisms could be detected. The poliovirus type 1 was the most sensitive, being inactivated within the first hour, whereas *C. albicans* was the most resistant, requiring more than 28 hr of composting for its inactivation. The data from this study indicated that aerobic composting of sewage sludge would destroy the indicator pathogens when a temperature of 60 to 70°C is maintained for a period of 3 days.

Methods Nos. 25, 51, 66, and 93

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Method: Not specified

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Method: Not specified

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APPENDIX A

OUT-OF-SCOPE LITERATURE CITATIONS

This section lists citations that were encountered during this study but were not actually used. The citations are organized into nine general categories, which are described in Section 1 as follows:

1. Land Application
2. Disinfection
3. Thermophilic processes
4. Other sludge treatment processes
5. Other sludges
6. Other organisms
7. Non-quantitative data
8. Review articles
9. Article not obtained

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APPENDIX B

ANALYTICAL METHODOLOGY

Analytical methods used to enumerate bacteria, viruses and parasites by the researchers cited in Sections 3, 4 and 5 are presented below.

Methods 1 to 20 describe procedures used to enumerate indicators bacteria; 21 to 51 describe procedures for pathogens.52 to 77 describe methods for enteric viruses, and 78 to 96 describe methods used to enumerate parasites.

Each procedure is cross-referenced by procedure number (at the beginning of each procedure and referenced in Section 6) and by author (at the end of each method).

METHOD NUMBER 1

Organism: Total Coliforms

Methodology: Most Probable Number technique by Standard Methods
Presumptive: Lactose broth[1].
Confirm: Brilliant green bile broth[2].
Completed: Eosin Methylene Blue Agar[3].

Standard Methods for the Examination of Water and Sewage Am. Publ. Health Assoc. NY, NY 8th ed., 1936. 11th ed., 1960. 14th ed., 1960. 19th ed., 1975.

Authors:	Rawn and Candell, 1950	(8th ed.) 1,2
	Cooke *et al.*, 1978	(14th ed.) 1,2
	Noland *et al.*, 1978	(13th and 14th ed.) 1,2
	Taylor *et al.*, 1978	(13th ed.) 1,2
	Burge and Cramer, 1974	(13th ed.) 1,2
	Gaby, 1975	(13th ed.) 1,2
	Cooper and Golueke, 1979	(13th ed.) 1,2
	Doyle, 1967	(11th ed.) 1,2,3
	Shook *et al.*, 1962	(10th ed.) 1
	Baxter et al., 1980	(14th ed.) 1, 2

METHOD NUMBER 2

Organism: Total Coliforms

Methodology: Most Probable Number Technique by Standard Methods

Presumptive: Lactose broth

Confirmed: Eosin Methylene Blue Agar

Standard Methods for the Examination of Water and Wastes, Amer. Publ. Health Assoc. NY, NY 9th ed. 1946

Author: Fuller and Litsky, 1950 (9th ed.)

METHOD NUMBER 3

Organism: Total Coliforms

Methodology: Most Probable Number Analysis as described in:

Walker, J.M. et al. Trench Incorporation of Sewage Sludge in Marginal Agricultural Land. U.S. EPA 600/2-75-034, Cincinnati, Ohio, 1975.

Author: Epstein et al., 1976

METHOD NUMBER 4

Organism: Total Coliforms

Methodology: Membrane Filter Technique by Standard Methods

Standard Methods for the Examination of Water and Waste Water. American Publ. Health Assoc. Wash., D.C. 14th ed. 1975

Authors:	Berg and Berman, 1980	(14th ed.)
	Jewell et al., 1980	(14th ed.)
	Noland et al., 1978	(13th and 14th ed.)
	Lue-Hing et al., 1974, 1977	(14th ed.)
	Bovay Engineering, 1975	(13th ed.)

METHOD NUMBER 5

Organism: Total Coliforms

Methodology: Serial dilutions were spread plated on M-Endo LES agar. Plates were incubated for 24 hr at 35°C.

Author: Dudley et al., 1980

METHOD NUMBER 6

Organism: Fecal Coliforms

Methodology: Most Probable Number Technique by Standard Methods.

Standard Methods for the Examination of Water and Wastes, Amer. Publ. Health Assoc. 14th ed. 1975
13th ed. 1971.

Authors:	LaConde et al., 1978a, b	(14th ed.)[1]
	Feige et al., 1975	(13th ed.)
	Noland et al., 1978	(13th & 14th ed.)
	Taylor et al., 1978	(13th ed.)
	Burge and Cramer, 1974	(13th ed.)
	Gaby, 1975	(13th ed.)
	Cooper and Golueke, 1979	(13th ed.)
	Baxter et al., 1980	(14th ed.)

1. Sample Pretreatment

Each 100 g sample of sludge was thoroughly mixed with 1 l of distilled, sterile water (plant samples were homogenized). The mixture was strained through a U.S. No. 6 sieve into a 1-gal disposable reinforced paper container. Solids retained on the sieve were discarded. The remainder was poured through a No. 5 standard sieve using a sterile wooden tongue depressor to "screen" the material through the sieve. Solids retained on the sieve were discarded. The remainder was aspirated with a "Millipore" or similar suction unit through 6- to 14-μ filters using approximately 133 ± ml (1/6 of total) of the mixture for each aspiration. The remainder was then aspirated through 0.45 μ filters to collect organisms.

METHOD NUMBER 7

Organism: Fecal Coliforms

Methodology: Most Probable Number Analysis as described in:

Walker et al. Trench Incorporation of Sewage Sludge in Marginal Agricultural Land U.S. E.P.A. 600/2-75-034, Cincinnati, Ohio, 1975.

Author: Epstein *et al.*, 1976

METHOD NUMBER 8

Organism: Fecal Coliforms

Methodology: Membrane Filter Technique by Standard Methods

Standard Methods for the Examination of Water and Waste Water. American Publ. Health Assoc. Wash., D.C. 14th ed. 1975 13th ed. 1971

Authors:	Berg and Berman, 1980	
	Jewell *et al.*, 1980	
	Farrell *et al.*, 1974	(13th ed.)
	Noland *et al.*, 1978	(13th and 14th ed.)
	Counts and Shuckrow, 1974	(13th ed.)
	Hinesly, 1974	
	Lue-Hing *et al.*, 1974, 1977	(14th ed.)
	Bovay Engineering, 1975	(13th ed.)
	Sacramento Area Consultants, 1979	
	Reeves, 1959	
	Kenner *et al.*, 1971	

METHOD NUMBER 9

Organism: Fecal Coliforms

Methodology: Serial dilutions were spread plated on m-FC agar. Plates were incubated at 35°C for 4-5 hrs then transferred to 44.5°C for 20 hrs.

Author: Dudley *et al.*, 1980

METHOD NUMBER 10

Organism: Fecal Streptococci

Methodology: Most Probable Number Technique by Standard Methods

Standard Methods for the Examination of Water and Wastes.
Amer. Publ. Health Assoc. 13th ed., 1971, 14th ed., 1975.

Authors:		
	LaConde *et al.*, 1978a,b	(14th ed.)[1]
	Feige *et al.*, 1975	(13th ed.)
	Cooper and Golueke, 1979	(13th ed.)

1. Sample Pretreatment

Each 100 g sample of sludge was thoroughly mixed with 1 l of distilled, sterile water (plant samples were homogenized). The mixture was strained through a U.S. No. 6 sieve into a 1-gal disposable reinforced paper container. Solids retained on the sieve were discarded. The remainder was poured through a No. 5 standard sieve using a sterile wooden tongue depressor to "screen" the material through the sieve. Solids retained on the sieve were discarded. The remainder was aspirated with a "Millipore" or similar suction unit through 6- to 14-u filters using approximately 133 ± ml (1/6 of total) of the mixture for each aspiration. The remainder was then aspirated through 0.45 u filters to collect organisms.

METHOD NUMBER 11

Organism: Fecal Streptococci

Methodology: Membrane Filter Technique by Standard Methods

Standard Methods for the Examination of Water and Waste Water. American Publ. Health Assoc. Wash., D.C. 14th ed. 1975, 13th ed. 1971

Authors:		
	Berg and Berman, 1980	(14th ed.)
	Jewell *et al.*, 1980	(14th ed.)
	Farrell *et al.*, 1974	(13th ed.)
	Noland *et al.*, 1978	(13th and 14th ed.)
	Lue-Hing *et al.*, 1974, 1977	(14th ed.)
	Bovay Engineering, 1975	(13th ed.)
	Sacramento Area Consultants, 1979	

METHOD NUMBER 12

Organism: Fecal Streptococci

Methodology: Pour plate technique

Pour plates are prepared using KF Streptococcus agar. Incubate at 35°C for 48 hours *or*:

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Pour plates are prepared using Pfizer Selective Enterococcus Agar. Incubate at 35°C for 18-24 hrs.

Standard Methods for the Examination of Water and Wastes Amer. Publ. Health Assoc. 13th ed. 1971

Authors: Counts and Shuckrow, 1974

METHOD NUMBER 13

Organism: Fecal Streptococci

Methodology: Serial dilutions were spread plated on m-Enterococcus agar. Plates for 48 hrs at 35°C.

Author: Dudley *et al.*, 1980

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METHOD NUMBER 14

Organism: *Escherichia coli*

Methodology: Modified most Propable Number Technique in MacConkeys Broth and tryptone broth. Incubated at 44°C.

Author: Krogstad *et al.*, 1975

METHOD NUMBER 15

Organism: *Escherichia coli* (ATCC 8739) suspended in compost

Methodology: Filter technique using Endo agar with confirmation in McConkeys nutrient medium and subsequently on Endo agar.

Author: Glathe *et al.*, 1972

METHOD NUMBER 16

Organism: *Escherichia coli*

Methodology: See Method Number 41

Author: Dudley *et al.*, 1980

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METHOD NUMBER 17

Organism: Enteric Bacteria

Methodology: Inoculated onto Endo agar.
Incubated for 24 hrs at 37°C.

Authors: Kampelmacher and van Noorle Jansen, 1972

METHOD NUMBER 18

Organism: Total Enterobacteria

Methodology: VRBM media as described by:

Mossle, D.A.A., Mengerink, W.H.J., and Scholts, H.H. A McConkey Type Agar Medium for the Selective Growth and Enumeration of All Enterobactericeae. J. Bact. 84: 381. 1962.

Author: LeClerc _et al._, 1970

METHOD NUMBER 19

Organism: Bacteriophage MS-2 seeded into an anaerobic digestor

Methodology: Elution: 25 ml sludge was homogenized for 30 sec. and adjusted to a pH 9 with NaOH.

Disinfection: 5 ml chloroform added, mixture stirred in an ice bath for 1 hr.

Concentration: Centrifuged at 4°C at 3000/rpm for 30 min. supernatant discarded, sediment resuspended.

Assay: Resuspended solids were assayed on the MS-2 host cell, E. coli C3000.

Authors: Bertucci _et al._, 1977
Lue-Hing _et al._, 1973

METHOD NUMBER 20

Organism: F^2 Bacteriophage seeded into lime (CaO), $FeCl_3$ and polymer treated sludge prior to vacuum filtration[1]. F^2 Bacteriophage seeded into liquid sludge[2].

Methodology: Elution: Sludge or sieved compost suspended in phosphated buffered saline (pH 7.0)[2]. Sludge suspended in phosphate buffered, mixed with beef extract (to make a 3.0% solution, and pH adjusted to 9.0 with NaOH.[2].

Resultant solution diluted in Tryptone Yeast Extract broth[1].

Resultant solution sonicated at 100W for 15 minutes then centrifuged at 10,000 g for 10 min followed by pH adjustment to 7.2[2].

Disinfectant: chloroform[1]

Assay: Assayed on *Escherichia coli* K-13 (ATCC No. 15766) by the overlay method described in: Bacteriophages, M.H. Adams, Interscience Publ., Inc. 1959[1, 2].

Authors: Burge and Cramer, 1974[2].
Cramer, 1975[1].
Baxter *et al.*, 1980[2].

METHOD NUMBER 21

Organism: *Salmonella* and *Shigella* spp.

Methodology: Standard Methods

Standard Methods for the Examination of Water and Wastes. Amer. Publ. Health Assoc., 14th ed. 1975.

Authors:
LaConde, *et al.*, 1978a,b.1
Jewell *et al.*, 1980
Reeves, 1959
Doyle, 1967 (11th ed.)
Shook *et al.*, 1962 (10th ed.)
Glathe *et al.*, 1972 (Inoculation study)

1. Sample Pretreatment:

Each 100 g sample of sludge was thoroughly mixed with 1 l of distilled, sterile water (plant samples were homogenized). The mixture was strained through a U.S. No. 6 sieve into a 1-gal disposable reinforced paper container. Solids retained on the sieve were discarded. The remainder was poured through a No. 5 standard sieve using a sterile wooden tongue depressor to "screen" the material through the sieve. Solids retained on the sieve were discarded. The remainder was aspirated with a "Millipore" or similar suction unit through 6- to 14-u filters using approximately 133 ± ml (1/6 of total) of the mixture for each aspiration. The remainder was then aspirated through 0.45 u filters to collect organisms.

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METHOD NUMBER 22

Organism: Salmonella and Shigella spp.

Methodology: None quantitative

Enrichment: Selenite F broth and Selenite brilliant green with sulfapyridine.
Incubated at 41.5°C for 18-24 hr.

Confirmed: Salmonella-Shigella agar
Bismuth sulfite agar
MacConkey agar
Incubated at 35°C for 18-24 hr

Completed: Inoculated into Triple sugar iron agar at 35°C for 24 hr.

Author serological identification performed.

Author: Gaby, 1975

METHOD NUMBER 23

Organism: Salmonella sp.

Methodology: None quantitative

Enrichment: Muller-Kaufmann tetrathionate broth.
Incubated for 20-48 hr at 430°C.

Confirmed: Brilliant green phenol red agar

Suspected colonies were subjected to further biochemical and serological examination.

Author: Kampelmacher and van Noorle Jansen, 1972

METHOD NUMBER 24

Methodology: Semi quantitative technique

Enrichment: Serial dilutions inoculated into Selenite broth to yield a final volume of 100 ml.
Tubes were incubated at 37°C for 24 hr.

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Presumptive: Serial dilutions of enrichment cultures were plated onto Brilliant green agar. Incubate at 42°C for 24 hr.

Confirmed: Pink colonies were subcultured to heart infusion agar and then to TSI and YIA slants.

Author: Dudley *et al.*, 1980

METHOD NUMBER 25

Organism: *Salmonella newport* seeded into raw sludge

Methodology: Enrichment: Inoculated into Gram negative broth. Incubated at 41.5°C for 6 hr.

Confirmed: Streaked onto Selenite brilliant green agar. Incubated at 37°C for 36 hr.

Completed: Inoculated into triple sugar iron agar. Incubated for 24 hr. at 37°C.

Further identification based on sludge agglutination.

Authors: Wiley and Westerberg, 1969

METHOD NUMBER 26

Organism: *Salmonella* sp.

Methodology: Most probable number technique

Sludge emulsified in phosphate buffered peptone and incubated overnight at 37°C. Supernatant passaged through:

Enrichment: Tetrathionate broth
42°C for 48 hrs.

Confirmed: Brilliant green agar and Deoxycholate-citrate agar[1]. Bismuth-Sulfide agar[2]. 37°C for 18-24 hr.

Suspect colonies inoculated onto triple sugar iron agar slants for incubation at 35-37°C for 18 hr.

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Positive samples tested for agglutination with polyvalent O&H antiser

Authors: Burge and Cramer, 1974[1]
Baxter *et al.*, 1980[2]

METHOD NUMBER 27

Organism: *Salmonella* and *Shigella* sp.

Methodology: Most Probable Number as described by Livingstone, 1965[1].

Enrichment: Selenite

Isolation: Lactose citrate desoxycholate

[1]. Livingstone, D.J. An Improved Method for Isolating Salmonella from Polluted Waters. Publ. Health, 2: 87-91, 1965.

Author: LeClerc *et al.*, 1970

METHOD NUMBER 28

Organism: *Salmonella* sp.

Methodology: Most probable number analysis as described by: Walker *et al.*. Trench Incorporation of Sewage Sludge in Marginal Agricultural Land. U.S. E.P.A. 600/2-75-034, Cincinnati, Ohio, 1975.

Author: Esptein *et al.*, 1976

METHOD NUMBER 29

Organism: *Salmonella* and *Shigella* sp.

Methodology: Most Probable Number Technique

Presumptive: 5 undiluted portions in GN broth.
Incubate for 24 hrs at 35°C.

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Confirmed:	Xylose lysine desoxycholate agar (XLD) and Hektoen enteria agar (HEA). Incubate at 35°C for 24 hrs. Triple sugar iron agar. Incubate at 35°C for 18 hr.
Completed:	Enterotubes. Incubate 24 hrs at 35°C.

Author: Sacramento Area Consultants, 1979

METHOD NUMBER 30

Organism: *Salmonella* and *Pseudomonas aeruginosa*

Methodology: Most probable number technique (with Membrane filtration)

Enrichment:	Dulcitol selenite broth (DSE) plus sodium acid selenite. Incubated at 40°C for 24-48 hrs.
Confirmed:	Xylose lysine desoxycholate agar. Incubated at 37°C for 24 hr.
Completed:	*Salmonella* inoculated into Kliger iron agar. *Ps. aeruginosa* inoculated into King A agar. Incubated at 37°C for 24 hr.

As described by: Kenner, B.A., and H.P. Clark. Detection and Enumeration of *Salmonella* and *Pseudomonas aeruginosa*. J. Wat. Poll. Control D. 46 (9): 2163-2171, 1974.

Authors:
Feige *et al.*, 1975
Farrell *et al.*, 1974
Noland *et al.*, 1978
Counts and Shuckrow, 1974
Lue-Hing *et al.*, 1974, 1977
Kenner *et al.*, 1971
Kenner and Clarke, 1974

METHOD NUMBER 31

Organism: *Salmonella* sp.

Methodology: Samples were centrifuged at 7000xg at 8°C for 30 min. The supernatant was discarded and the sediment was resuspended in selenite broth. Incubated at 43°C for 24 hrs. Cultures were streaked onto brilliant green and Lactose sucrose urea agar.

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All plates incubated at 37°C and examined at 24 and 48 hrs.

Isolated colonies were identified serologically.

Author: Danielsson, 1977

METHOD NUMBER 32

Organism: *Salmonella*

Methodology: Alley-Malaney membrane filter technique.
Enrichment: Tetrathionate broth 24 hr.
Selection: Bismuth sulfite broth 48 hr.

Author: Cooke *et al.*, 1978

METHOD NUMBER 33

Organism: *Salmonella typhimurium* (NVH 2632) seeded into compost.

Methodology: Compost material was mixed with modified k-tetrathionate broth.
Incubated at 41°C for 24-72 hr.

Positive tubes were subcultivated onto Bromothymol blue lactose agar.

Author: Krogstad and Gudding, 1975

METHOD NUMBER 34

Organism: *Salmonella typhosa* (inoculated)

Methodology: Spot plate technique.

4, 0.02 ml spots per dilution at 2 dilutions were made on each.
Incubate 24 hr at 37°C.
Bacterial counts made from the sum of the four spots.
Detection limit = 250/ml.

Media: Tryptone glucose extract agar, Eosin methylene blue agar, Bismuth sulfite agar, and Brilliant green agar.

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Author: McKinney *et al.*, 1958

METHOD NUMBER 35

Organism: *Shigella* sp.

Methodology: Semi quantitative technique

Enrichment: Serial dilutions inoculated into GN broth to yield a final volume of 100 ml. Tubes were incubated for 24 hrs at 37°C.

Presumptive: Serial dilutions from each enrichment were spread on XLD agar. Plates were incubated at 37°C for 24 hr.

Confirmed: Red or colorless colonies were transferred to heart infusion agar slants and then to TSI agar and motility indole ornithine media. Positive isolates were confirmed with antisera.

Author: Dudley *et al.*, 1980

METHOD NUMBER 36

Organism: *Mycobacterium* sp.

Methodology: Sample preparation: Sludge was mixed (10:1) with diluted Zephiran chloride. After a treatment period of 35 to 40 min. at room temperature, the mixture was diluted.

Samples were spread plated onto Middlebrooks 7H11 agar with oleic-albumin-dextrose-catalase enrichment, modified with fungizone.

Plates were incubated at 37°C in a 5% CO_2-in. air atmosphere and observed over a 4 week period.

Isolated colonies were observed for acid fast bacilli.

All non-chromogenic colonies were subcultured to Lowenstein-Jensen medium to test for niacin production.

Author: Dudley *et al.*, 1980

METHOD NUMBER 37

Organism: Mycobacterium tuberculosis

Methodology: Samples were treated with H_2SO4 to make a 15% solution. After 30 min. digestion, the solution was centrifuged. The resultant percipitate was resuspended in a salt solution, filtered through a membrane filter and washed with a salt solution until a neutral pH was obtained.

Filter deposits were inoculated onto egg culture medium, with and without glycerine, incubated for up to 6 weeks.

Author: Muller, 1959

METHOD NUMBER 38

Organism: Mycobacterium sp.

Methodology: As described by: Nguematcha, R. Etude de la Flore Mycobacterienne des eaux Residuaires. Pouvoir d'arret des Stations d'epuration et devenir des Mycobacteries au cours des traitements subis parles boues. These doctorat d'Etat Pharmacie, Lille 1969.

Author: LeClerc et al., 1970

METHOD NUMBER 39

Organism: Mycobacterium tuberculosis var hominis H37RV seeded into sludge

Methodology: Sludge sample was treated with Bradosol (a quarternary ammonium compound) for 20 min. which results in a 70-80% recovery. Serial dilutions were plated onto Dubos Oleic Acid Agar.

Author: Heukelekian and Albanese, 1956

METHOD NUMBER 40

Organism: Mycobacterium tuberculosis var hominis inserted into a windrow.

Methodology: Add 15-20 ml. of 3 percent sodium hydroxide (NaOH) to 2 grams of inoculated material and adjacent compost material.

Agitate the mixture for 5 minutes.
Incubate the mixture for 30 minutes at 37°C.
Decant the supernatant fluid and filter through sterile cheesecloth.

Centrifuge the supernatant fluid for 15 minutes at high speed in sealed sterile tube.

Neutralize the sediment with normal HCl containing 0.01 percent by volume phenol 6.4-6.8. Decant all but approximately 1 ml. Streak a loopfull of sediment onto Lowenstein-Jensen media.

Place 1/2 ml. of the solution on the surface of Lowenstein-Jensen medium.

Incubate 37°C--examine weekly for 8 weeks.

If typical colonies occur, the niacin test was used to determine if the colonies were *Mycobacterium tuberculosis* var *hominis*.

Authors: Morgan and McDonald, 1969

METHOD NUMBER 41

Organism: *Aeromonas hydrophila*

Methodology: Serial dilutions of sludge samples were plated on both MacConkey and XLD agars.
Plates were incubated at 37°C for 24 hrs.

Discrete colonies were streaked to MacConkey agar for isolation.

Subsequent identification involved oxidase testing (Kovacs procedure) and the use of the API20E system. (Analytab products Inc., Plainview, N.Y.

Density of the bacteria was calculated on the basis of occurrence per dilution.

Author: Dudley *et al*., 1980

METHOD NUMBER 42

Organism: *Clostridium perfringens*

Methodology: Most Probable Number technique

Sample Preparation: samples were untreated (vegetative
Confirmed: Positive tubes were transferred to litmus milk tubes.
Incubated for 1-3 days at 37°C.

Author: Dudley *et al*., 1980

METHOD NUMBER 43

Organism: *Klebsiella pneumoniae*

Methodology: See Method Number 41

Author: Dudley *et al*., 1980

METHOD NUMBER 44

Organism: *Proteus morganii*

Methodology: See Method Number 41

Author: Dudley *et al*., 1980

METHOD NUMBER 45

Organism: *Pseudomonas aeruginosa*

Methodology: See Method Number 41

Author: Dudley *et al*., 1980

METHOD NUMBER 46

Organism: *Yersinia enterocolitica*

Methodology: See Method Number 41

AUTHOR: Dudley *et al*., 1980

METHOD NUMBER 47

Organism: Staphylococcus sp.

Methodology: Dilutions were spread onto mannitol salt agar plates which were incubated at 37°C for 24 to 48 hrs.

All gram postive cocci that exhibited a yellow tone of fermentation on MSA were counted as Staphylococci.

Author: Dudley et al., 1980

METHOD NUMBER 48

Organism: Pathogenic fungi

Methodology: As described in:
Cooke, W.B. Fungi in Polluted Water and Sewage. II. Isolation technique. Sewage and Industrial Wastes. 26: 661-674, 1954

Authors: Cooke and Kabler, 1955

METHOD NUMBER 49

Organism: Pathogenic fungi

Methodology: Add compost to sterile physiological saline. Shake to suspend and cntrifuge for 15 minutes at 2500 rpm. Decant supernatant ad mix sediment with penicillin (10,000 units) and streptomyin (10 mg).

After 20 minues standing at room temperature, inoculate 3 white Swiss mce intraperitoneally each with 0.5 ml of sediment. After weeks, sacrifice mice. Remove and mince spleen and portion o liver.

Use small porions to inoculate 2 tubes of Sabouraud's agar (I) and 2 tubs of Sabouraud's agar plus cycloheximide (0.5 mg/ml) and choromycetin (0.05 mg/l) (II).

Incubate for weeks at 25°C, examining weekly. Prepare smears of likly colonies and identify by standard cultural methods. Als inoculate I and II with portion of the concentrated sedment. Incubate for 6 weeks at 25°C and identify by cultual characteristics.

Author: Gaby, 1975

METHOD NUMBER 50

Organism: *Aspergillus fumigatus*

Methodology: Compost was shaken with 0.01% Triton X-100. Dilutions prepared were plated on Oxgall-antibiotic Agar and incubated at 25 and 44°C.

Author: Millner *et al.*, 1977

METHOD NUMBER 51

Organism: *Candida albicans* seeded into sludge

Methodology: Presumptive: Dilutions of sludges were prepared in Sabouraud Dextrose Broth.

Primary isolation was made by the pour plate technique on a modification of Pagano Levin Base as described by: Stedham, M.A., D.C. Kelly, and E.H. Coles. Modified Pagano Levin Medium to Isolate Candida Species. Appl. Microb. 14: 525-528, 1966.

Incubate 5 days at room temperature.

Confirmed: Organisms were confirmed by their production of chlamydospores on Rice Extract Agar.

Authors: Wiley and Westerberg, 1969.

METHOD NUMBER 52

Organism: Enteroviruses

Methodology: Elution: Glycine buffer and Tween 80 added and pH adjusted to 10.5. Mixture was stirred and then sonified.

Concentration: Centrifuged at 9400xg at 4°C for 30 min. Supernatant adjusted to pH 7.5 and freon 113 added.

Supernatant removed and centrifuged then dialyzed against polyethylene glycol.

Assay: Resultant solution was assayed on Buffalo Green Monkey kidney cells at 37°C for 8 days and examined for PFU.

Authors: Metropolitan Sanitary District of Greater Chicago, 1979
Lue-Hing et al., 1974, 1977
Wellings et al., 1976: Elution: pH 9.0 + beef extract

Concentration: A similar concentration technique was used, with the elimination of freon.

METHOD NUMBER 53

Organism: Enteroviruses

Methodology: Elution: Not performed

Concentration: 100 ml of sludge was concentrated to 1.5 ml using $CaHPO_4$ adsorbent.

Assay: Concentrate was assayed, using the MPN method, on Primary Monkey Kidney tube cultures.

Incubated for 2 weeks at 37°C followed by blind passage with the medium of each tube and another 2 week incubation. Most Probable Number of cytopathogenic units counted.

Virus strains were identified by the HAI (hemagglutinating inhibition) and neutralization tests.

Author: Palfi, 1972

METHOD NUMBER 54

Organism: Enteroviruses

Methodology: Elution: Equal volumes of sludge and double strength tryptose phosphate broth were homogenized in a blender for 5 min at a high speed.

Concentration: Solids were removed by centrifugation and discarded.
Supernatant assayed.

Assay: Supernatant incubated on HeLa cultures. PFU counted for 3 days.

Author: Moore *et al.*, 1978

METHOD NUMBER 55

Organism: Enteroviruses

Methodology: Elution: Mix sludge sample with 0.05 M glycine (pH 11.5) for 30 s.

Concentration: Adjust pH to 3.5, centrifuge at 16,000 xg for 10 min.
Elute sediment with fetal calf serum.

Disinfection: Pass supernate through 3.0, 0.45, and 0.22 m filters.
Elute filters at pH 11.5.

Assay: Inoculated onto BGM cells by PFU.

Authors: Hurst *et al.*, 1978
Bitton *et al.*, 1980

METHOD NUMBER 56

Organism: Enteroviruses

Methodology: Elution: a) Beef extract: A 10% solution of beef extract af pH 7.0 was added and the mixture was homogenized by vigorous shaking for 2 hrs. Sludge particles were centrifuged down at 16,000 g for 30 min at 4°C. The supernatant was adjusted to pH 7.2 and decontaminated.

b) Acid precipitation followed by elution with beef extract: As described in Lydholm and Nielsen (1979).

c) Alum precipitation followed by elution with tris buffer: The sample was treated with $Al(SO_4)18\ H_2O$ as described in Lydholm and Nielsen (1979).

d) Direct inoculation: The sample was diluted and decontaminated (not specified).

Decontamination and Assay: As described in: Lydholm, B., and Nielsen, A.L. Methods for the Detection of Virus in Wastewater, Applied to Samples from Small Scale Treatment Systems. Water Research, 14: 169-173, 1979.

Author: Nielsen and Lydholm, 1979

METHOD NUMBER 57

Organism: Enteroviruses

Methodology: As described by:
Kelly, S.M., M.E. Clarke, and M.B. Coleman. Demonstration of Infectious Agents in Sewage. Amer. J. Publ. Health, 1955, 45, 1438-1446.

Author: LeClerc et al., 1970

METHOD NUMBER 58

Organism: Enteroviruses

Methodology: Sample preparation: Samples were homogenized in a Waring blender for 30 s, then centrifuged at 1500 g. The supernate was discarded and the sediment retained.

Elution: The sediment was resuspended in:
a) 3% casein hydrolysate (pH 7.2-7.5)
b) lactalbumin hydrolysate (pH 7.2-7.5)
c) 3% beef extract (pH 7.2-7.5)
d) fetal calf serum (pH 7.2-7.5)
The suspension was shaken for 30 min in a wrist action shaker.

Concentration: Centrifugation at 1500 g for 20 minutes.

Disinfection: Supernate was filtered through a 0.22 um membrane filter.

Assay: The filtrate was then inoculated onto BS-C-1 cells and incubated for 3 weeks. Cultures were examined for cytopathic effect.

Authors: Sattar and Westwood, 1976

METHOD NUMBER 59

Organism: Enterovirus

Methodology: From each sample, the following materials were inoculated into cell cultures.

a) An etherized sample as described by: Lund, E., C.E. Hedstrom, W. Jantzen. Occurrence of enteric viruses in waste water after activated sludge treatment. J. Water Poll. Contr. Fed. 41: 169-174, 1969.

b) A precipitate and supernate after centrifugation at 9000 rev min^{-1} (13,200 g) for 30 min.

Assay: Aliquots were inoculated onto Hela cells. The adsorption period was kept at 2 hrs. Cultures were incubated at 37°C for 7 days. All negative or suspected cultures were carried through two blind passages.

Author: Lund, 1973.

METHOD NUMBER 60

Organism: Enterovirus

Methodology: Elution: Not performed

Concentration: Not performed

Disinfection: Etherization

Assay: Aliquots were inoculated into Hela and trypsinized into monkey kidney cell cultures and incubated for one week. Up to 2 blind passages were carried out on negative or suspected cultures.

Isolates were identified by the use of neutralization tests.

Author: Lund, 1970

METHOD NUMBER 61

Organism: Enteric Viruses (enteroviruses and reovirus)

Methodology: An ill-defined sedimentation step was followed by:

Elution: Sludge sample was mixed with Phosphate buffered saline (pH 7.4) plus 10% fetal calf serum. The suspension was homogenized for 30 sec in a Servall Omni-mixer.

Concentration: Following 3 or more freeze-thaw cycles, the sediment was discarded and supernate retained.

Disinfection: Supernatant treated with 5% chloroform or passed through a 0.2 um filter.

Assay: The extract was assayed on Primary Monkey Kidney Cells for greater than 24 hours. Viruses were detected as either cytopathic effects (in liquid media) or PFU.

Author: Cliver, 1975

METHOD NUMBER 62

Organism: Enterovirus and Reovirus

Methodology: Elution: Sludge mixed with fetal calf serum (pH 7.2) and shaken in a wrist-action shaker for 30 min at room temperature.

Concentration: Centrifugation at 1500 g for 20 min.

Disinfection: Supernate filtered through a 0.22 um filter.

Assay: Filtrate inoculated onto BS-C-1 cells and incubated for up to 3 weeks. Cultures were examined for cytopathic effect.

Authors: Sattar and Westwood, 1979

METHOD NUMBER 63

Organism: Enteric viruses

Methodology: Elutriation: 1% bovine albumin, no further specifications[1].

Assay: host cell not specified[1].

[1] For a complete description, refer to:

Diaz et al. Public Health Aspects of Composting Combined Refuse and Sludge and of the Leachates Therefrom. Final Report for the State of Calif. Solid Waste Management Board. College of Engineering, Univ. of Calif., Berkeley, June 1977.

Authors: Cooper and Golueke, 1979[1]

METHOD NUMBER 64

Organism: Enteric Viruses

Methodology: Elution: Sludge was mixed with 10% beef extract in McIlvaine buffer and sufficient citric acid to yield a pH of 7.

Mixed on a magnetic stirrer for 30 min.

Concentration: Centrifuged at 10,000 xg for 30 min. Sediments discarded

Disinfection: Supernate was filtered through 0.45 um filter.

Assay: Filtered supernate assayed by PFU technique on BGM cells.

Author: Berg and Berman, 1980

METHOD NUMBER 65

Organism: Enteric viruses

Methodology: Sample collection: cheesecloth swabs were suspended in the flowing sludge for 48 hrs.

Elution: Approximately 100 ml of swab expression were reduced to 10 ml by adsorbing on 5 g of Dowex-1 resin, to which bovine albumin had been added, and eluting twice with beef extract broth.

Disinfection: Atibiotics and ether were added.

Assay: Virus wee assayed for Monkey kidney epithelium and Hela cels. Plates were examined for Plaque Forming Unit aftr 7-10 da incubation for 33-35°C.

Author: Kelly and Sanderson, 1959

METHOD NUMBER 66

Organism: Poliovirus type 1 seeded into sterile sewage sludge

Methodology: Elution: Sample containing virus was adjusted to pH 7.2 after the addition of NaCl, Polyethylene glycol, and sodium dextran sulfate.
Mixture was homogenized for 1 hr.

Concentration: Mixture transfered to a separatory funnel for 24 hours.

Disinfection: Supernate from above was ether treated.

Assay: Triplicate aliquots were assayed on stationary tube FL cell tissue culture at 35°C and examined for cytopathic effects.
Virus suspensions were also challenged with antiserum in neutralization tests.

Author: Wiley and Westerberg, 1969

METHOD NUMBER 67

Organism: Poliovirus Type 1, strain CHAT (seeded into sludge and aerated for 15 min prior to digestion)

Methodology: Decontamination: Not performed

Elution: Not performed

Concentration: Samples of digested sludge were centrifuged for 5 min at high speed.
Supernatant used for direct assay, solids resuspended in phosphate buffered saline, for direct assay.

Assay: Sample dilutions inoculated onto Hela cells.
Overlain with modified Eagles medium plus antibiotics incubated for 2 days at 37°C.
PFU counted.

Author: Sanders _et al._, 1979

METHOD NUMBER 68

Organism: Poliovirus (Type 1 Sabin) inoculated into a sewage sample.

Methodology: Sample Preparation:

Fraction 1: Sewage mixed with virus. Sewage with virus mixed with $Ca(OH)_2$ to a pH of 11.5. The sample was transferred to an Imhoff cone and allowed to settle for 1 hour.

Fraction 2: Supernatant removed and pH-adjusted to 7.2. The sludge from the Imhoff cone was centrifuged at 5,000 x g for 20 min and the supernatant discarded. The sediment was resuspended in distilled water and adjusted to pH 7.2.

Fraction 3: It was again centrifuged and the resultant supernate was collected. The sediment from the second centrifugation was resuspended in 10% fetal calf serum (pH 7.2) and shaken for 15 min at room temperature.

Fraction 4: The mixture was centrifuged and the resultant supernate collected.

Elution: Fetal calf serum was mixed with Fractions 1,2, & 3

Disinfection: Samples were filtered through a 0.45 um membrane filter.

Assay: The fractions were tested, diluted, and undiluted in EBSS and assayed on BS-C-1 cells (temperature, time not specified).

Author: Sattar et al., 1976

METHOD NUMBER 69

Organism: Poliovirus, type 1 (CHAT) seeded into dewatered and partially composted digester solids

Methodology: Elution: Sludge sample was centrifuged at 20,000 xg for 20 min.
The solids pellet was suspended at 10 g (dry weight) per 200 ml of 3% beef extract and homogenized with a polytron mixer.

The eluant was subject to one of 3 treatments:
a) brought to pH 10 (34% recovery)

b) brought to pH 9 and disrupted by sonic treatment for 2 min (30% recovery)

c) disrupted by sonic treatment without pH adjustment (47% recovery)

Each treatment was followed by centrifugation at 20,000 xg for 20 in. The resultant supernate was adjusted to pH 7.

Treatment (C) was judged to be the most efficient process.

Concentration: Eluates were concentrated by using the organic flocculation technique (60-65% recovery) as described by:

Katzenelson, E.B., B. Fattal, and T. Hostovesky. Organic Flocculation: An Efficient Second Step Concentration Method for Detection of Viruses in Tapwater. Appl. Environ. Microbio. 32: 638-639, 1976.

Detoxification: Dithizone in chloroform was added to concentrate and resultant mixture was centrifuged for 15 min. at 40,000 x g. Aqueous phase was aerated for 10-15 min. and residual dithizone was complexed by addition of 0.05 ml. of 0-1% $CaCl_2$.

Assay: Assayed by plaque method on BGMK cells.

Author: Glass et al., 1978

METHOD NUMBER 70

Organism: Seeded Poliovirus type 1 (Mahoney)

Methodology: Elution: Not performed

Concentration: Not performed

Disinfection: Not performed

Assay: Aliquots were inoculated onto HeLa monolayers. Viruses were adsorbed for 45 min. at 37°C.

Cultures were incubated at 37°C in 2.5% CO_2 for 2 days.

Author: Moore *et al.*, 1975

METHOD NUMBER 71

Organism: Poliovirus inoculated into wastewater

Methodology: Elution: Samples were eluted with distilled deionized water and with tryptose phosphate broth

Concentration: Not specified

Assay: Samples were inoculated onto HeLa cells.
Temperature, time of incubation not specified.

Author: Malina *et al.*, 1975

METHOD NUMBER 72

Organism: Enterovirus, seeded Polio-type 2

Methodology: Elution: The sludge samples were adjusted to 0.1 M tris (hydroxy methyl) aminomethane (Tris-CCl pH 7.5), 0.2 M NaCl, 5% W/V glycerol, and 10% V/V F.BS and stirred for 10 min at 22°C.
Mixture was centrifuged at 20,000 xg rpm for 30 min at 4°C.
This procedure was performed 2 times on the sediment with supernatant fractions combined.

Concentration: The combined supernatants were centrifuged at 45,000 xg rpm for 3 hours at 4°C.
The resultant pellets were resuspended by sonication in 1.0 ml minimal essential growth medium containing 10% FBS and 20 mM HEPES.

Assay: Assays were performed on RD cells, BGM cells, VERO cells, WI-38 cells, LLC-MK_2 cells, and HeLa, and DEF cells.
The inoculum was adsorbed for 1.0 at 37°C.
Plaques were counted 48 to 105 hr post inoculation.

Authors: Nath and Johnson, 1979

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METHOD NUMBER 73

Organism: Poliovirus, 1, strain Sabin
Coxsackie, A9, strain Griggs
Coxsackie, B4, strain JVB
Echovirus, 11, strain Gregory
Seeded into an anaerobic digester.

Methodology: Elution: Not performed

Concentration: Not performed

Assay: Ten-fold dilutions of sludge were made in maintenance medium with antibiotics and inoculated onto Primary Rhesus Monkey kidney cultures.
Allowed to adsorb for 4 hours.
Cultures incubated for 7 days at 37°C and examined for cytopathic effect.
Virus titer calculated by the Reed Muench method.

Authors: Bertucci *et al.*, 1977
Lue-Hing *et al.*, 1973

METHOD NUMBER 74

Organism: Poliovirus type 1, strain CHAT; type 1, strain Mahoney, and type 2, strain 712-Ch-ZaB, Coxsackie B1, Reovirus type 3.
Seeded into sludge and mixed for 15 min at room temperature

Methodology: Decontamination: Not performed

Elution: Sodium dodecyl sulfate to 0.1% followed by sonication at room temperature for 2 min.

Concentration: Centrifuged at 18,000 xg for 20 min or not performed.

Assay: Inoculated onto Hela cells and allowed to absorb for 30 min at 37°C (Polio, Coxsackie), 2h (Reovirus).

Overlain with Eagles medium and incubated for 24 h (type 2), 40 h (type 1), and 70h (Reovirus).
PFU counted.

Authors: Ward and Ashley 1976
Ward and Ashley 1977a, b; 1978a, b, c; 1979
Ward, 1978

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Ward and Ashley, 1978a, b, c
Ward and Ashley, 1979

METHOD NUMBER 75

Organism: Polio type 1 (Sabin strain)
Echo type 6
Coxsackie type B4
Seeded into sludge supernate

Methodology: Elution: Not performed

Concentration: Not performed

Assay: Ten-fold dilutions were inoculated onto Vero African Green Monkey kidney cell monolayers in Micro Test II Tissue Culture plates.
Allowed to adsorb for 2 hours at 25°C.
Incubated at 37°C in a CO_2 incubator
The $TCID_{50}$ was determined by the method of Reed and Muench.

Author: Fenters *et al.*, 1977

METHOD NUMBER 76

Organism: Echo, Polio, Coxsackie virus Inoculated into sludge

Methodology: Elution: Samples were treated with EBSS (pH 9.5) containing 2-10% fetal calf serum and homogenized in a Sorvall Omnimix in 3, 2 min spurts, cooling materials in an ice bath throughout.

Concentration: The homogenate was centrifuged at 6000 rpm for 20 min.

Disinfection: Chloroform treatment.

Assay: Samples were assayed on a mixed cell culture of African Green monkey kidney cells (AGMK) and diploid human embryonic lung cells (WI-38 strain).
Cultures were incubated at 37°C for up to 10 days and examined for cytopathic effects. Negative samples were rerun.

Author: Subrahmanyan, 1977a, b

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METHOD NUMBER 77

Organism: Coxsackie virus type B3 (seeded into an anaerobic digester)

Methodology: Decontamination: 2:1 ether to sludge for 3 - 12 h at 4°C
Residual ether removed by bubbling air.

Elution: Not performed

Concentration: Not performed

Assay: Sample inoculated onto HeLa cells and allowed to adsorb for 3 hrs, followed by rinsing with maintenance media.
Incubate 7 days at 37°C.
50% TCID determined.

Author: Eisenhardt *et al*., 1977.

METHOD NUMBER 78

Organism: *Ascaris lumbricoides*

Methodology: Pre-treatment of sample: All compost was milled to pass a 1-5 cm mesh sieve. A 20 g test sample was suspended in 200 ml of warm sterile water (40°C) containing 0.5 g of detergent. This suspension was then homogenized in a Waring blender for 45 seconds.

Procedure:

1. Weight out 30 g of the homogenized suspension into a 100 ml beaker.

2. Sieve by passing the whole contents of the beaker through copper wire gauze (15 mesh cm^{-1}) into an evaporating basin. Rinse beaker using 20 ml of sterile tap water passing the washings through the sieve.

3. Transfer the fluid in the evaporating basin to four 40 ml centrifuge tubes allowing approx. 12.5 ml per tube. Top up each tube with sterile tap water and spin at 2000 rev per minute for 1.5 min. Discard supernatant. Repeat using sterile tap water, until supernatant is clear. Discard final supernatant.

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4. Add 7 ml of 10% formalin (1 vol. 40% formaldehyde diluted with 9 vol water) to each a tube and emulsify. Add 2 ml of ether. Shake vigorously for 30 s.

5. Centrifuge at 2000 rev min $^{-1}$ for 1.5 min.

6. A layer of debris will have accumulated at the interface between the two liquids. Loosen it by passing a swabstick gently around the circumference of the tube. Discard the supernatant.

7. Stir up the deposit and examine microscopically.

8. Transfer the residue retained on the copper wire mesh (normally discarded) to a 100 ml beaker and immerse in 20 ml of sterile tap water plus 4 to 5 drops of liquid detergent and then stir with a swabstick for 2 min. Resieve the whole and centrifuge the liquid for 1.5 min at 2000 rev min^{-1}. Pour off the supernatant and treat the deposit further as from step 4 onwards.

Approximately 100% recovery of seeded ova was obtained with this method.

Author: Steer _et al._, 1974.

METHOD NUMBER 79

Organism: _Ascaris lumbricoides_

Methodology: Sludge samples were examined for _Ascaris_ ova by zinc flotation and by quantitative Stoll counts.

Author: Bond, 1958

METHOD NUMBER 80

Organism: _Ascaris lumbricoides_ ova suspended in sludge

Methodology: Eggs were removed from sludge and incubated for 35 days at 28°C in distilled water.

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They were then examined for development.

Author: Schatzle, 1969

METHOD NUMBER 81

Organism: *Ascaris lumbricoides* ova inoculated into sludge

Methodology: Ova were obtained from the uteri of 27-36 cm. *A. lumbricoides*

After exposure to an inactivation environment, the water was decanted and 0.1N H_2SO_4 was again added to the tubes as the embryonation media. The ova were incubated for 21 days at 30°C on a roller drum which revolved at a speed of 1 revolution in 5 minutes and 15 seconds.

Following embryonation, the tubes were shaken vigorously to evenly disperse the ova throughout the suspension. Samples were extracted from the tube and placed in a McMasters Chamber for counting under the Leitz microscope. Four counts were made from each sample with each count representing 0.1 ml of ova suspension. Counts included embryonated and non-embryonated ova; non-fertilized ova were disregarded. Ova were considered embryonated only if embryonation had reached the larval stage and the larva was clearly visible within the ovum. Similar counts were made on control (non-exposed) tubes and the data normalized to establish the control embryonation at 100%.

Authors: Brandon, 1978
Brannen *et al.*, 1975

METHOD NUMBER 82

Organism: *Ascaris lumbricoides* ova suspended in sludge digester

Methodology: Periodically, the containers were removed from the digester and ova placed in a humid environment at 30°C. After 2-3 days they were examined for development.

Author: Stroh, 1961

METHOD NUMBER 83

Organism: *Ascaris lumbricoides* ova

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Methodology: Viability determined by isolating and culturing the ova at 37°C in a medium consisting of 5% formalin in physiological saline. Culturing and microscopic examinations were repeated every two weeks.

Authors: Verrannan, 1977
Kriege, 1964

METHOD NUMBER 84

Organism: *Ascaris* ova

Methodology: Compost was mixed with formol saline and filtered through gauze. Ether was added to the filtrate and shaken and the resultant mixture was centrifuged and the sediment examined microscopically.

Author: Kriege, 1964

METHOD NUMBER 85

Organism: *Ascaris lumbricoides* var *suum* seeded into anaerobically digested sludge

Methodology: Ova were stripped from the uteri of adult worms and inoculated into sludge.

Each sample was held in shaken flasks at 38°C for 21 days. After 21 days, formalin was added to all flasks to make a 1% solution and they were aerated for an additional 21 days by bubbling air.

Samples from each were examined microscopically and the percent embryonation determined.

Authors: Fitzgerald and Ashley, 1977

METHOD NUMBER 86

Organism: *Ascaris lumbricoides* var *suum*

Methodology: Ascaris ova from uteri inoculated into sludge

0.035 ml sample applied to a glass slide without concentration for microscopic enumeration.

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Author: Newton *et al*., 1949
Reyes *et al*., 1963

METHOD NUMBER 87

Organism: Ova of *Ascaris suis* obtained from adult nematodes collected from a slaughter house and seeded into sludge.

Methodology: Approximately 0.3 g of sludge solids was blended with 10 ml of 7 x 1 full strength detergent, Limbo Scientific, Inc., and distilled water. The resultant slurry was passed through a 40 mesh tyler screen and the filtrate collected and allowed to settle for 2-3 hours.

The settled solids were subsampled by weight and then repeatedly mixed with 10% 7x, shaken and centrifuged (1 min at 3500g) until the supernatant was clear.

The resultant solids were mixed with 10% 7x and added to the top of Shaetler's solution gradient tubes (10, 30, 33, 40, 80% solution).

The gradient tubes were then centrifuged for 10 minutes at 1,000g. The 30, 33, and 40% layers were removed and mixed with 10% 7x, then resuspended in 30% Shaetler's solution.

The sample was counted in a Sedgewick Rafter cell.

Author: Baxter *et al*., 1980

METHOD NUMBER 88

Organism: *Taenia saginata* seeded into sludge

Methodology: Ova were obtained from stools of infected individuals and inoculated into sludge:

Samples were filtered and centrigued for the recovery and evaluation of the conditions of the ova. Viability was determined by microscopic comparison with normal eggs. Changes included blackening of eggs and disorganization of their internal structure.

Author: Newton *et al*., 1949

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METHOD NUMBER 89

Organism: *Taenia saginata* seeded into sludge

Methodology: The sludge-egg mixture was put through a 90 mesh sieve by gently tapping the mesh while water was applied. The sieve residue was discarded and the total filtrate centrifuged for 10 sec. at 3,000 r.p.m. (radius 15 cm). The supernatant was poured off and the sedimented matter resuspended in water. It was then poured into an Imhoff cone which held 1,500 mls. of water and which had been adapted to run off the settled matter. The particles which settled during the first 0 to 15 secs. were run off and rejected while the 15 sec. to 10 min. fraction was collected.

The criteria for determining the viability of the tapeworms eggs consist of: (1) an in vitro test which stimulates hatching and activation of the contained tapeworm egg embryos, and (2) the microscopical appearance of the eggs. Hatching and activation is accomplished by placing the eggs in various solutions of intestional enzymes. Confirmation of the vitro tests was obtained by feeding eggs from the test batch to susceptible calves to determine their infectivity.

Author: Silverman and Guiver, 1960

METHOD NUMBER 90

Organism: Encysted *Trichinella spiralis* larvae obtained from the diaphragms, tongues, and masseter and skeletal muscles of infected laboratory rats.

Methodology: Raw sludge was mixed with rat tissue containing encysted larvae (5 larvae/ml) for both batch and continuous digester studies. The continuous digester was fed sludge containing 5 larvae/ml daily while the batch digester 2as fed unseeded sludge.

Daily draw offs from digesters were fed to rats by stomach pumps.

Seven or 8 weeks after the rats had been given 'draw offs' they were sacrificed and the tissue from the tongue, masseter, and diaphragm were removed and placed in a trichinoscope compression slide and examined for larvae.

Authors: Fitzgerald and Prakasam, 1978

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METHOD NUMBER 91

Organism: Parasites

Methodology: Mix compost within solution.

(a) Direct mount: Allow solution to settle for 30 min. Prepare iodine mount using 0.05 ml of sediment for direct microscopic examination.

(b) Brine flotation method: Mix suspension and strain through wire mesh funnel into centrifuge tube (50 ml). Wash mesh with saline (15-265 ml). Centrifuge for 1 to 2 minutes at 2000 rpm. Decant supernatant and resuspend sediment in saline. (Save 10 ml for suspension for formalin-ether method). Centrifuge remaining 10 ml as before, decant supernatant, resuspend in brine. Transfer into shell vial, fill with brine to lip and cover with sludge. Avoid overflow and airpockets. After 15 minutes remove slide, fit with cover glass, seal edges with Vaspar and examine microscopically.

(c) Formalin-ether sedimentation: Centrifuge 10 ml suspension from brine flotation method. Decant supernatant. Add 10 ml of 10% formalin, mix and allow to stand for 5 minutes. Add ether (3 ml), shake for 20 seconds and centrifuge at 1500 rpm for 1 to 2 minutes. Four layers are formed. Decant top 3 layers and prepare an iodine mount from the bottom sediment.

Author: Gaby, 1975

METHOD NUMBER 92

Organism: Parasites

Methodology: Qualitative microscopic examination

Author: Farrel *et al.*, 1974

METHOD NUMBER 93

Organism: Parasites

Methodology: Sludge was concentrated by centrifugation. Parasites were obtained by $ZnSO_4$ sedimentation followed by Alcohol-acid and ether treatment. The resultant solution is examined microscopically for viable ova.

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Authors: Jewell *et al.*, 1980
Keller, 1951
Theis *et al.*, 1978[1]
LaConde *et al.*, 1978a, b
Keller and Hide, 1959
Wiley and Westerberg, 1969[2]

[1] Quantification of ova were made using standard McMasters Technique.

[2] *Ascaris lumbricoides* ova inoculated into sludge. No Alcohol or ether treatment performed.

METHOD NUMBER 94

Orgamism: Parasites

Methodology: Nematode ova were isolated from sludge by means of the sugar flotation procedure.

Extracted ova were placed in 1.5% formalin and aerated for 22°C for 21-28 days. At the end of this period, ova were removed and examined for development.

Author: Arther and Fitzgerald, 1977

METHOD NUMBER 95

Organism: Parasites

Methodology: The sludge was sieved through two series of wire mesh screen in a coarse-fine sequence. The final screened material was allowed to gravity sediment for 3-45 minutes and then the water layer was decanted. Aliquots of the sludge were run by a standard sedimentation and flotation technique using saturated zinc sulfate and 10 percent buffered formalin respectively. Six (6) 15 ml test tube samples were run for each sludge sample, three with the flotation and three with the sedimentation procedure. Slides were made from the test tube samples and examined for parasitic protozoa and helminths under a compound microscope. Permanent photomicrograph slides were also made and stained with a trichrome method for identification of protozoan parasites.

Enumeration is made using the MacMaster's slide technique.

Author: Sacramento Area Consultants, 1979

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METHOD NUMBER 96

Organism: Parasites

Methodology: About 250-300 ml of 1% 7X (an anionic detergent) is added to 50-100 ml of the sludge sample. The mixture is seived through 10, 20, 50, 100, and 150 gauge seives and the resultant filtrate allowed to settle for at least one hour. The sediment produced is centrifuged 2 to 3 times at 2000 rpm for at least 5 minutes. The packed volume of sediment resulting from the centrifugation is mixed with $ZnSO_4$ solution (1.20 specific gravity) and centrifuged. The resultant supernate is again centrifuged and the sediment washed, then mixed with alcohol acid solution, followed by ether. After this mixture is centrifuged, the resulting sediment is examined microscopically at 100X magnification.

Author: Reimers *et al.*, 1980

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